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U.S. Army Environmental Center

FINAL ANTI-AIRCRAFT ARTILLERY RANGES SAMPLING AND ANALYSIS PLAN, FORT SHERIDAN, ILLINOIS

August 27, 1999

Distribution unlimited, approved for public release

Prepared for:

U.S. ARMY ENVIRONMENTAL CENTER
Base Closure Division
Aberdeen Proving Ground, Maryland 21010-5401

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This sampling and analysis plan presents the planned surface water and sediment sampling and analysis to be conducted at the surface water intakes and anti-aircraft artillery ranges in Lake Michigan. The potential to encounter unexploded ordnance is also addressed. Surface water and sediment samples will be analyzed for explosives constituents.

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Final
Anti-Aircraft Artillery Ranges Sampling and Analysis Plan, Fort Sheridan, Illinois

Prepared for:
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List of Abbreviations and Acronyms

AAA	anti-aircraft artillery
ANL	Argonne National Laboratory
BEC	BRAC Environmental Coordinator
BRAC	Base Realignment and Closure
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act
CFR	Code of Federal Regulations
DA	Department of the Army
DoD	Department of Defense
DQOs	data quality objectives
EOD	explosive/ordnance/disposal
FR	Federal Register
GPS	Global Positioning System
HASP	Health and Safety Plan
HMX	Octahydrotetranitrotetrazocine-High Melting Explosive
HPLC	high performance liquid chromatograph
IDW	investigative derived waste
IRP	Installation Restoration Program
KAT	Katalyst Analytical Technologies, Inc.
MDL	method detection limit
mm	millimeter
MS	matrix spike
NCP	National Contingency Plan
QAPP	Quality Assurance Project Plan
OQAPP	Overall Quality Assurance Project Plan
OSHA	Occupational Safety and Health Administration
PA	Preliminary Assessment
QC	quality control
QA/QC	quality assurance/quality control
RI	remedial investigation
SAP	sampling and analysis plan
SARA	Superfund Amendments and Reauthorization Act
SOP	Standard Operating Procedure
TM	Technical Memorandum
TNT	tetryl, trinitrotoluene
USACE	U.S. Army Corps of Engineers
USEPA	U.S. Environmental Protection Agency
UXO	unexploded ordnance

1.0 Project Description

The U.S. Army Installation Restoration Program (IRP) was designed to identify and control or abate constituent migration resulting from past operations at Department of the Army (DA) installations. The IRP is the DA's environmental response under the authority of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) of 1980, as amended by the Superfund Amendments and Reauthorization Act (SARA) of 1986. As delegated by Executive Order 12580, the DA is responsible for determining response actions, consistent with the National Contingency Plan (NCP) [40 Code of Federal Regulations (CFR) Part 300], necessary for the abatement of contamination resulting from releases of hazardous substances at its installations.

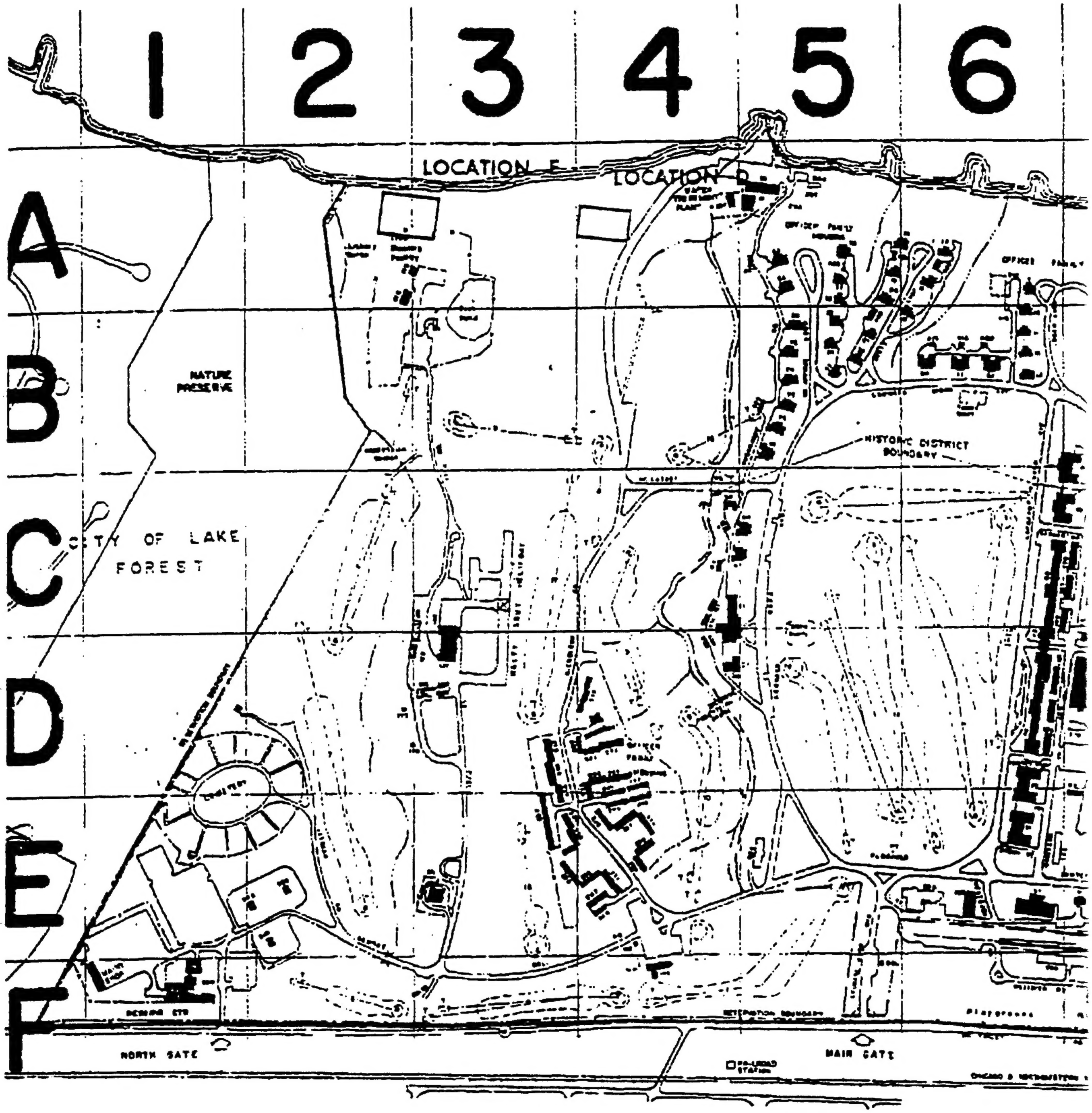
1.1 Background Information

1.1.1 Investigative and Site History

Preliminary assessments (PAs) of Fort Sheridan, conducted in 1982 and 1989, identified several former anti-aircraft artillery (AAA) firing points along Fort Sheridan [Gross *et al.*, 1982; Argonne National Laboratory (ANL), 1989]. AAA training occurred at Fort Sheridan from 1930 through the 1950s. AAA training commenced with the arrival of the 61st Coast Artillery. Five locations were considered suitable for firing positions for AAA at Fort Sheridan. These five locations are shown in Figure 1-1. Location A was the original firing point, but, because of complaints from local residents, location B became the primary firing location [U.S. Army Corps of Engineers (USACE), 1995]. Other sources indicate that locations C and D were also used as firing points (ANL, 1989).

The vast majority of firing was conducted against towed aerial targets. An archives search found evidence that ammunition used at the AAA ranges consisted of 37 millimeter (mm), 40 mm, 3 inch, 90 mm, and 120 mm projectiles (USACE, 1995). These projectiles consisted primarily of machined iron or steel casings and contained explosive fillers. Explosive fillers for the majority of these sizes of ordnance would have been tetryl, trinitrotoluene (TNT), black powder, or 50/50 ammonium nitrate and TNT [Complete Round Chart No. 5981, 1944 and 1945; War Department Technical Memorandum (TM) 9-1904, 1944; TM 9-1901, 1950; TM 9-1300-203, 1967]. Small amounts of brass, aluminum or zinc-lead alloy may have been used in the fuses of these projectiles. An analysis of the ranges of these various projectiles indicates that the majority of unexploded rounds would be from 3.7 miles to 10.6 miles from shore, with a decreasing potential of rounds out to 15.4 miles (Appendix A) (USACE, 1995).

Initial sampling efforts have been conducted at Fort Sheridan in relation to the potential for unexploded ordnance (UXO) and related explosives compounds to be present in Lake Michigan. Two sediment



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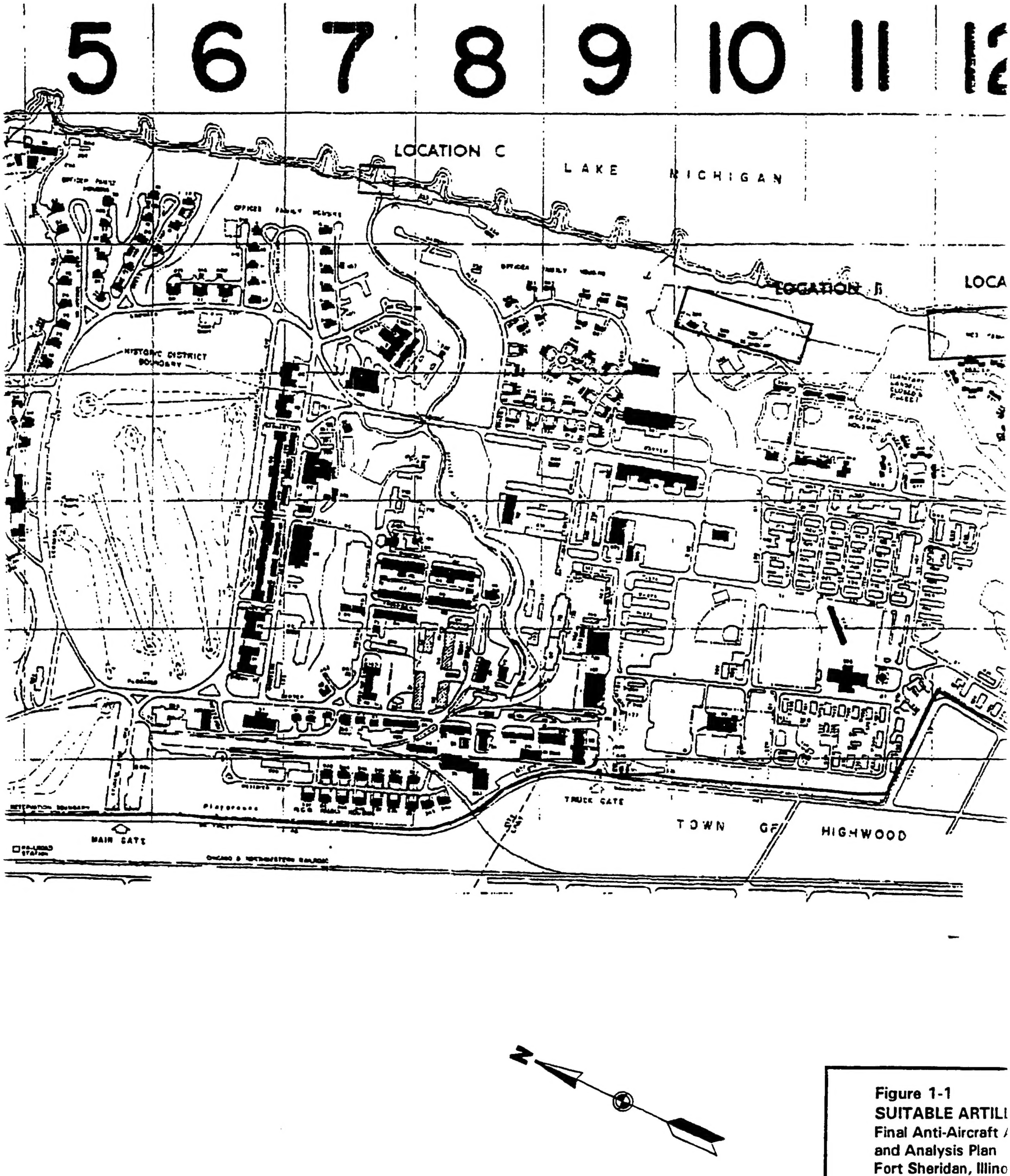

Figure 1-1
SUITABLE ARTILI
Final Anti-Aircraft /
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Fort Sheridan, Illino

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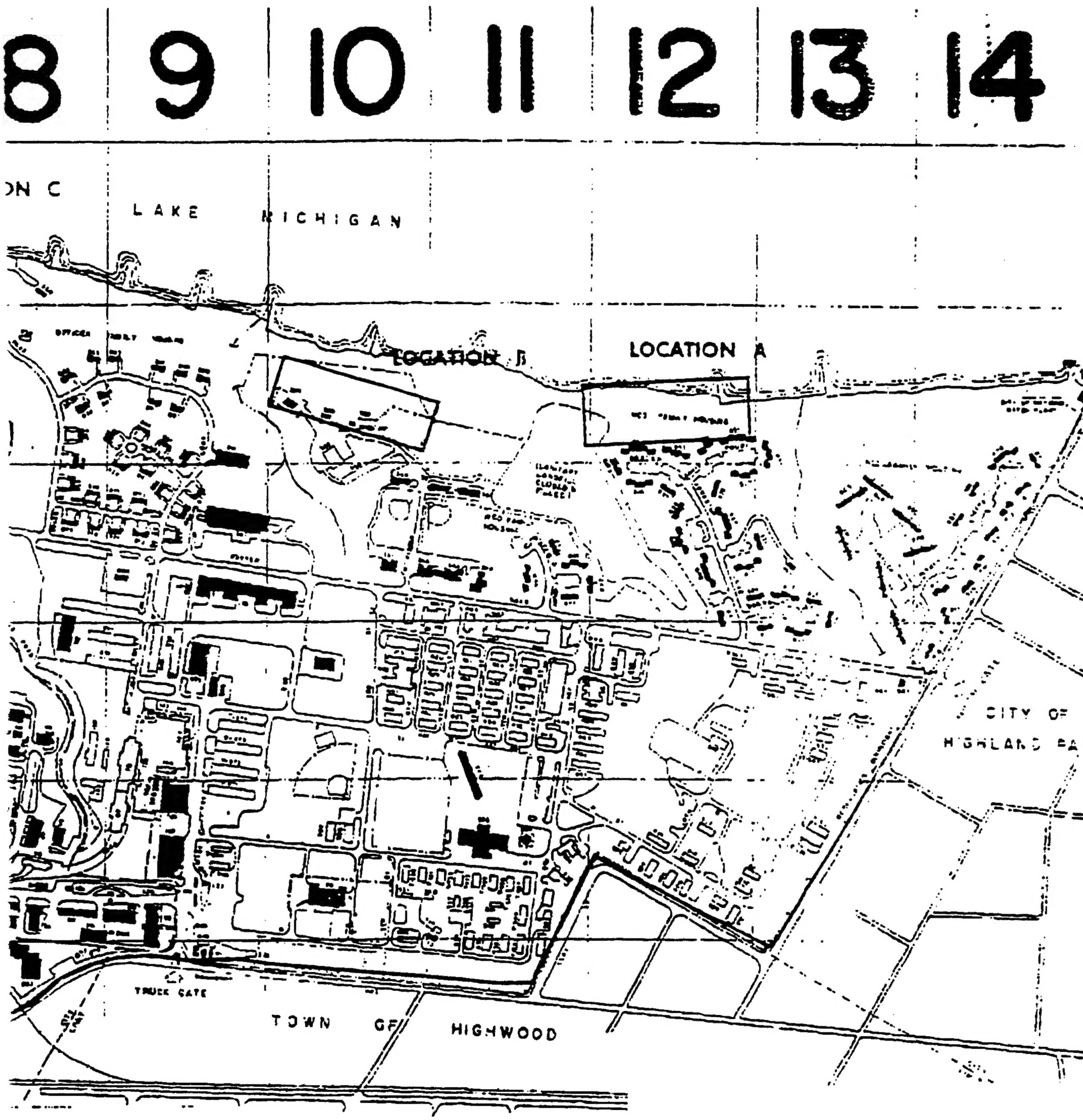

MAP 4

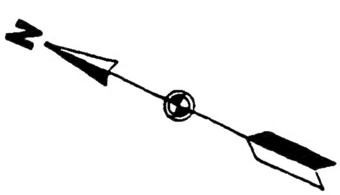

Figure 1-1
SUITABLE ARTILLERY FIRING POSITIONS
Final Anti-Aircraft Artillery Ranges Sampling and Analysis Plan
Fort Sheridan, Illinois

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grab samples were collected in approximately 3 feet of water in the area between Janes and Hutchinson Ravines, just east of firing point D. A low concentration (just above the detection limit) of 1,3-dinitrobenzene was detected in one of the sediment samples collected. Given the proximity of this sampling location to the shore and to a former ordnance disposal location, however, this detection is not likely related to the AAA ranges. This sampling effort is discussed in the Final Remedial Investigation (RI)/Baseline Risk Assessment for the Ravines and Beach Area Study Areas (QST, 1998).

A total of 20 offshore sediment samples were also collected at various locations along the Fort Sheridan shoreline along transects oriented perpendicular to the shoreline at distances of 30 feet and 70 feet. These sediment samples were collected to help evaluate the impact on Lake Michigan, if any, of surface water runoff and other types of discharge (e.g., groundwater) from Fort Sheridan. Six samples were collected offshore from the Lake Forest Nature Preserve; six samples were collected offshore from Landfill #7; four samples were collected offshore south of Shenck Ravine and near the southern boundary of Fort Sheridan; and the remaining four samples were collected offshore from the mouths of Janes, Hutchinson, Bartlett, and Van Horne Ravines. No explosives constituents were detected in any of the sediment samples. Three surface water samples were also collected during the offshore sediment sampling. One sample was collected offshore from the Lake Forest Nature Preserve; one sample was collected offshore from Landfill #7; and one sample was collected offshore at the southern boundary of Fort Sheridan. Octahydrotetranitrotetrazocine-High Melting Explosive (HMX) was detected below the method detection limit (MDL) in the surface water samples collected at the southern boundary of Fort Sheridan. This sampling effort is discussed in the Draft Final RI Report for the Department of Defense (DoD) Operable Unit (SAIC, 1999).

Four raw (untreated) water samples have also been collected, one at the Highwood water treatment plant and three at the Highland Park water treatment plant. None of these samples contained detectable concentrations of explosives compounds.

Additionally, in response to a citizen petition, U.S. Environmental Protection Agency (USEPA) completed its own PA of the AAA ranges. USEPA's PA assumed that the lake sediments in the vicinity of water plant intakes have been affected and, therefore, determined that further investigation of the AAA ranges was warranted (Muno, 1998). Pursuant to Executive Order 12580, the DA has requested that this sampling and analysis plan (SAP be developed for the AAA ranges. As a result, the Base Realignment and Closure (BRAC) Environmental Office has requested that a scope of work be prepared to further evaluate the nature and extent of potential explosives constituents in surface water and sediment near local Lake Michigan municipal water intakes and sediment in the AAA training impact zone. The purpose of this sampling is to further assess whether or not chemical constituents in artillery fired at the former AAA ranges have impacted or have the potential to impact Lake Michigan,

a valuable ecological resource as well as a local source of drinking water. The Army determined that further investigation of these ranges is appropriate to address anticipated requirements of the forthcoming Range Rule (see below) and to address community questions about the potential impacts of these ranges.

On September 26, 1998, the DoD issued a proposed rule that identifies a process for evaluating appropriate response actions on closed, transferred, and transferring military ranges (Range Rule) [Federal Register (FR), Volume 62, Number 187, Page 50795 (62FR50795)]. This proposed Range Rule contains a process that is not inconsistent with CERCLA and is tailored to the special risks posed by military munitions and military ranges.

1.1.2 Scientific Literature Research

As part of this investigation, a literature search was conducted on the potential fate of explosives compounds in the Lake Michigan underwater environment. This research focused on the degradation or breakdown of explosive compounds and on Lake Michigan sediment transport. These two factors have the potential to affect the detectability of AAA range constituents.

Research on the environmental fate of explosives indicates these compounds can break down. Once dissolved in water, TNT and tetryl readily decompose by interaction with water (hydrolysis) and/or by interaction with sunlight (photolysis). This decomposition can occur within hours or days. Conversely, TNT breakdown products form tightly bound complexes with sediments that are resistant to biodegradation (Walsh, 1990; Darrach and Chutjian, 1997; Naval Explosive Ordnance Disposal Technology Center, 1988; Layton *et al.*, 1987; Zappi, 1998). Thus, explosives constituents present in sediment are expected to degrade more slowly than explosives constituents dissolved in water. For this reason, chemical analysis must include TNT breakdown products. Additional information on the environmental fate of TNT and tetryl is provided in Appendix B.

The subsurface environment in Lake Michigan, including bottom sediments, is dynamic. Waves and currents in the lake are directly related to wind. Sediment distribution in Lake Michigan is primarily determined by wind-driven surface waves (Chang-Hee and Hawley, 1998). Lake currents have a well-established counter-clockwise pattern around the southern basin of the lake for most of the year. These currents affect the distribution of sediments, resulting in a general southward migration of sediments (Edgington and Robbins, 1990; Lineback and Gross, 1972). In addition, the Illinois coast of Lake Michigan is exposed to waves approaching from either the northeast or southeast quadrants. The predominance of northeasterly waves adds to the net southward transport of sediment (Foyle *et al.*, 1998). During a major storm event, wind-driven currents can affect the lake bottom to a depth of 25 to 30 meters (82 to 98 feet) (Chang-Hee and Hawley, 1998; Booth, 1994; Berkson *et al.*, 1975).

Therefore, there is some concern regarding sediment sampling for explosives constituents contained in UXO in such a dynamic environment, in addition to the fact that the most recent AAA training occurred in the early 1950's (USACE, 1995). However, studies of UXO in underwater environments indicate that UXO can last a long time in an underwater environment (at least 50 years) and can release their contents at a very slow rate through corroded pinholes, joints, or screw threads (Darrach and Chutjian, 1997). Therefore, if UXO are present in Lake Michigan offshore from Fort Sheridan, even though the sediments are constantly dispersing, a constant source of explosive constituents from an item of UXO may be detectable through sediment sampling.

1.2 Project Objective

The objective of the following scope of work is to define the nature and extent (if any) of potential explosives constituents in sediment and surface water adjacent to the water intakes and in sediment in the AAA impact zone. This scope of work will permit conclusions to be drawn regarding the presence of explosives near the water intakes and in the impact zone, and corresponding potential risks (if any) to human health and/or the environment. Because the Army and other services of the DoD (and perhaps non-military entities) historically conducted artillery training over Lake Michigan, an attempt was made in this SAP to limit, as much as possible, the scope of this study to evaluate the potential effects of Fort Sheridan AAA training on the lake.

2.0 Site Management

Site management activities support the data collection activities and include mobilization, site access and control, documentation, field instrumentation, decontamination, and control and disposal of investigative derived waste (IDW) (if any). The following subsections present the salient points summarized from their counterparts in the Overall Quality Assurance Project Plan (OQAPP) (ESE, 1995). The reader is referred to Section 2.0 of the OQAPP for a discussion of the project management structure. Unless otherwise discussed and specified, the work conducted under this study will be performed in strict accordance with the protocols and procedures included in the OQAPP and the Health and Safety Plan (HASP).

2.1 Mobilization

The following activities will be performed at Fort Sheridan as part of mobilization:

- Command post setup, including office and sampling equipment (boat, etc.) staging areas and communications. The location of the field/sample management office, Building 379, is shown in Figure 2-1.
- Field team orientation.
- Field team health and safety meeting.
- Check in, regularly coordinate and hold field schedule discussions with the Fort Sheridan BRAC Environmental Coordinator (BEC).
- Coordination of sampling efforts with the Highland Park and Highwood Water Treatment plants.

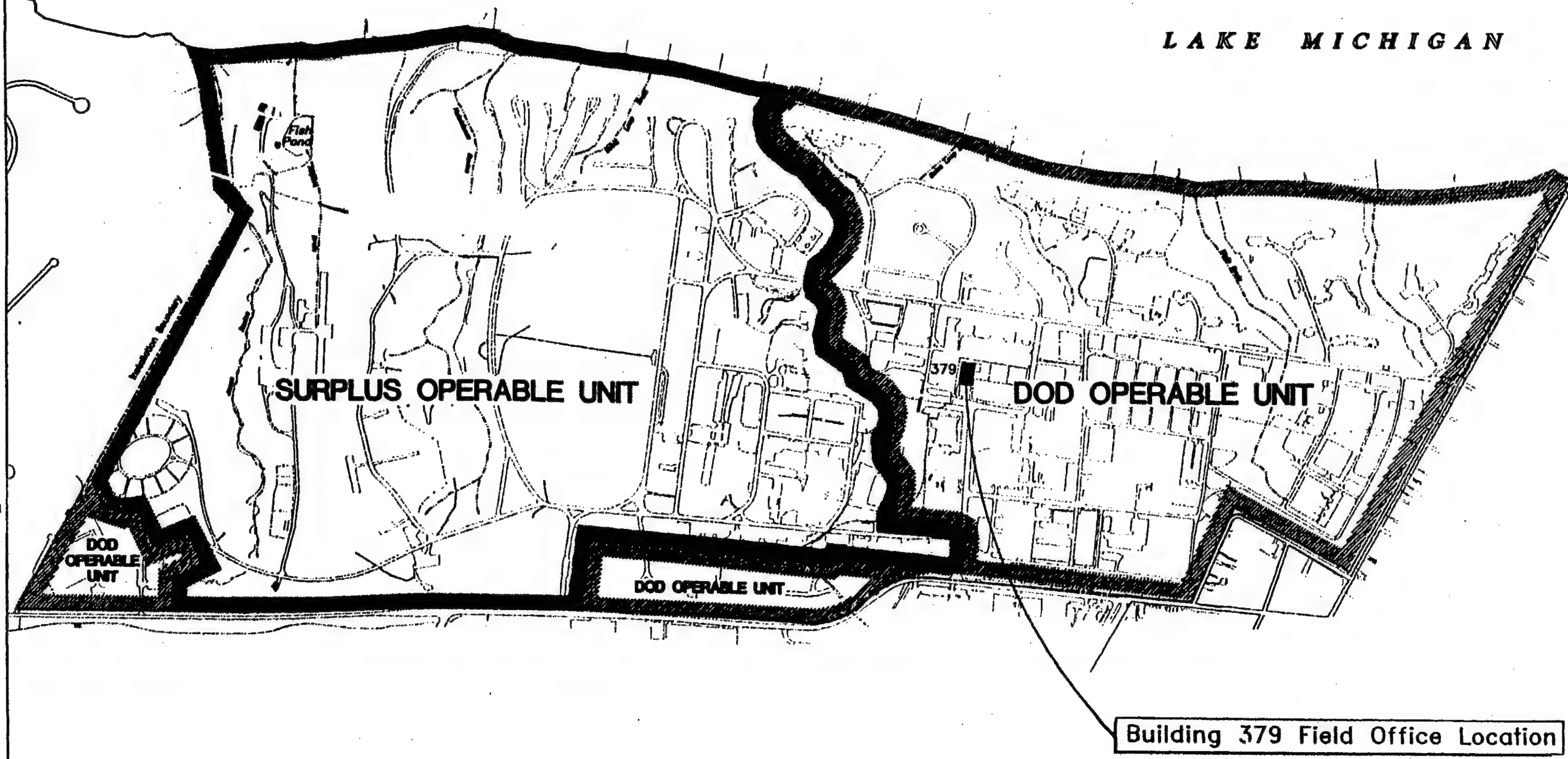
2.2 Site Access and Control

Fort Sheridan is an open installation and access to most study areas is unrestricted. Written access permission for any restricted areas will be obtained from, or through, the BEC prior to conducting any field work in these areas.

Field equipment left on-site overnight will be locked, if possible, in a trailer or vehicle.

2.3 Documentation

The reader is referred to Section 4.1.2 of the OQAPP for discussions of record keeping requirements and procedures that will be followed. Records of field activities performed at Fort Sheridan will be kept on-site during field operations. Particular items that will be kept on-site include the OQAPP,



Adapted from an aerial survey by Air Survey Corporation, Sterling, Virginia. Date of photography: 12/6/95.

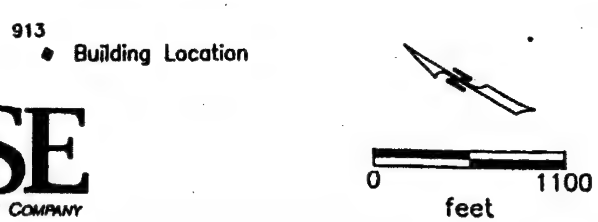


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Figure 2-1
FIELD OFFICE LOCATION
Final Anti-Aircraft Artillery Ranges Sampling and Analysis Plan
Fort Sheridan, Illinois

HASP, daily field logs, and certifications that contractor employees have current Occupational Safety and Health Administration (OSHA) training in Hazardous Waste Operations according to 29 CFR 1910.120.

2.4 Field Instrumentation

The reader is referred to Section 6.1 of the OQAPP for details of field instrumentation and calibration requirements. A list of equipment anticipated to be used during the implementation of this SAP is included as Appendix C. Sample collection techniques and materials not discussed in the OQAPP are described in Section 5.7.2 and 5.8.2 of this document.

2.5 Decontamination

Decontamination procedures will conform to the requirements discussed in Section 4.12 of the OQAPP.

2.6 Control and Disposal of IDW

It is anticipated that little or no IDW will be generated in this study. The only potential IDW to be generated is minor amounts of detergent water, isopropanol, etc. involved with the decontamination of stainless-steel sampling spoons/trowels, stainless-steel bowls, and/or a petite ponar dredge or Eckman dredge. Because of the diminimus quantities of liquid IDW that will be generated, this liquid will be containerized in 5-gallon lidded buckets and transported to Fort Sheridan for discharge into the sanitary sewer system.

3.0 RI Data Collection

This section includes descriptions of information gathering methods required to meet the site-specific data quality objectives (DQOs). Since this information has already been adequately presented in the OQAPP, the reader is referred to Sections 1.0 (specifically Table 1-1) and 4.0 of the OQAPP for pertinent discussions of DQOs and investigatory techniques, respectively. Sections 5.0, 6.1, and 11.1 of the OQAPP discuss sample custody procedures, field instrumentation calibration, and field instruments, respectively. The site-specific field investigation programs that will be implemented during this study are described in Section 5.0 of this document.

The characteristic of completeness of collected data, as defined in Section 3.4 of the OQAPP, is a measure of the amount of valid data obtained compared to the total data obtained. The expected minimum level of completeness to be achieved for each analyte for the field sampling effort and laboratory analyses for the Fort Sheridan RI as a whole is 90 percent.

4.0 Chemical Analysis Program

Sample holding times, container requirements, and preservation techniques are described in detail in Section 4.13 of the OQAPP. Specific chemical analyses and quality control (QC) sampling requirements to be used for this study are included in Section 5.0. The estimated number of QC samples required for the work to be performed under this study is summarized in Table 4-1.

4.1 Laboratory Analytical Procedure

The chemical analysis for the work described in Section 5.0 of this document is limited to explosives constituents by USEPA Method 8330. USEPA Method 8330 is a high performance liquid chromatograph (HPLC) procedure for the analysis of nitroaromatics and nitramines (SW-846). USEPA Method 8330 covers the same parameters as the explosives method (EXP1-S/W) presented in the OQAPP and has similar MDLs and reporting limits (refer to Section 7.0 of the OQAPP). Method 8330 is provided in Appendix D along with Method 8000B, Determinative Chromatographic Separations.

Samples will be analyzed by Katalyst Analytical Technologies, Inc. (KAT). KAT's reporting limits for Method 8330, as well as the Standard Operating Procedure (SOP) for Method 8330, have been provided previously as part of the Landfills 6 and 7 Interim Remedial Action OQAPP Addendum (Stone and Webster, 1999) (OQAPP Addendum) and are provided here as Appendix E. The laboratory will include the reporting limits with the analytical results.

4.2 Calibration Procedures and Frequency

The laboratory will follow the standard procedures addressed in their laboratory Quality Assurance Project Plan (QAPP) and outlined in the OQAPP Addendum.

4.3 Internal Quality Control Checks

The laboratory will follow the standard procedures addressed in their laboratory QAPP and outlined in the OQAPP Addendum.

4.4 Calculation of Data Quality Indicators

Laboratory results will be assessed for compliance with required precision, accuracy, completeness, and sensitivity. These parameters are defined in Section 12 of the OQAPP. The laboratory will

follow the standard procedures addressed in their laboratory QAPP and outlined in the OQAPP Addendum.

4.5 Corrective Actions

The laboratory will follow the standard procedures addressed in their laboratory QAPP and outlined in the OQAPP Addendum.

4.6 Data Management and Validation

Data validation will be performed on all of the analytical data discussed in this SAP. Validation will be performed in accordance with the USEPA National Functional Guidelines (USEPA, 1994a and 1994b). Selected data from 10 percent of the complete set of sample data will be subjected to a full comprehensive data validation. The remaining 90 percent will be subjected to a definitive data validation. Comprehensive data packages will be requested on 20 percent of the samples analyzed to ensure that adequate documentation is available to support those samples selected for comprehensive data validation. Validation will be performed in ESE's St. Louis office per the National Functional Guidelines.

4.7 Preventative Maintenance

The laboratory will follow the standard procedures addressed in their laboratory QAPP and outlined in the OQAPP Addendum.

4.8 Performance and System Audits

The laboratory will follow the standard procedures addressed in their laboratory QAPP and outlined in the OQAPP Addendum.

4.9 QC Reports to Management

The laboratory will follow the standard procedures addressed in their laboratory QAPP and outlined in the OQAPP Addendum.

Table 4-1. Quality Assurance/Quality Control (QA/QC) Program, Anti-Aircraft Artillery Ranges Water Intake SAP, Fort Sheridan, Illinois

			Number of QA/QC Samples			
Sampled Media	Estimated Number of Field Samples	Analytical Parameters for Field Samples	Duplicates*	Field/ Equipment Blanks†	Trip Blanks	Matrix Spikes & M.S. Dups.†
Surface Water	18	Explosives	3	--	--	1
Sediment**	36	Explosives	4	2	--	2

* Number of duplicate samples figured on 10 percent of total number of samples for each medium and analyte list.

† Number of field/equipment blank and matrix spike/matrix spike duplicate samples figured on 5 percent of total number of samples for each medium and analyte list.

** Additional samples will be collected if UXO are present.

5.0 Scope of Work

The scope of work consists of collecting surface water and sediment samples near the water intakes in Lake Michigan, collection of raw water samples at the Highland Park and Highwood treatment plants, and collection of sediment samples in the AAA impact zone in Lake Michigan. The water intake locations are presented in Figure 5-1 and the AAA impact zones is presented in Figure 5-2. Any significant field modifications to this Scope of Work will be approved by the BRAC Cleanup Team prior to implementation, if possible.

5.1 Water Intake Sampling

The location of each water intake will be verified using Global Positioning System (GPS). None of the water intakes are marked in the field. Therefore, topographic maps, engineering plans, or coordinates for the intakes will be obtained and used to locate the intakes with GPS. A Trimble Navigation real-time differential GPS with data logger will be utilized to locate the intakes and sampling points. U.S. Coast Guard digital GPS signals will be utilized to provide real-time differential correction of GPS signals. This will increase the GPS location accuracy to ± 5 feet.

The location of the intake will be verified with the GPS before sampling begins. The subsequent intake sampling stations will be determined by GPS. A surface water and a sediment sample will be collected at each station. The intake will not be disturbed in any way during sampling. Following the sampling effort, each of the sampling stations will be located on an appropriate scale figure via the GPS coordinates.

Water intake surface water and sediment sampling will be conducted by scuba-certified personnel. Each of the scuba divers will have current explosive/ordnance/disposal (EOD) and commercial diver certifications. A Work and Safety Plan for underwater UXO operations will be prepared by UXB International, Inc. and provided to the field team prior to sampling. Divers will be used to collect the samples in the event any UXO are present around the intakes. Although these water intakes are outside the impact zone, the PA conducted by USEPA assumes the source of explosives constituents is proximal to the intakes (Muno, 1998). Therefore, in order to verify the presence or absence of a source or sources proximal to the intakes, divers will be used to collect the intake surface water and sediment samples. Surface and/or underwater electronic sensing equipment will be used to locate UXO near the surface water intake. The presence of the UXO will be verified visually by the diver. If UXO are found near intakes, then additional sediment samples will be collected next to the UXO item. In any event, no fewer than eight surface water and eight sediment samples will be collected from each intake.

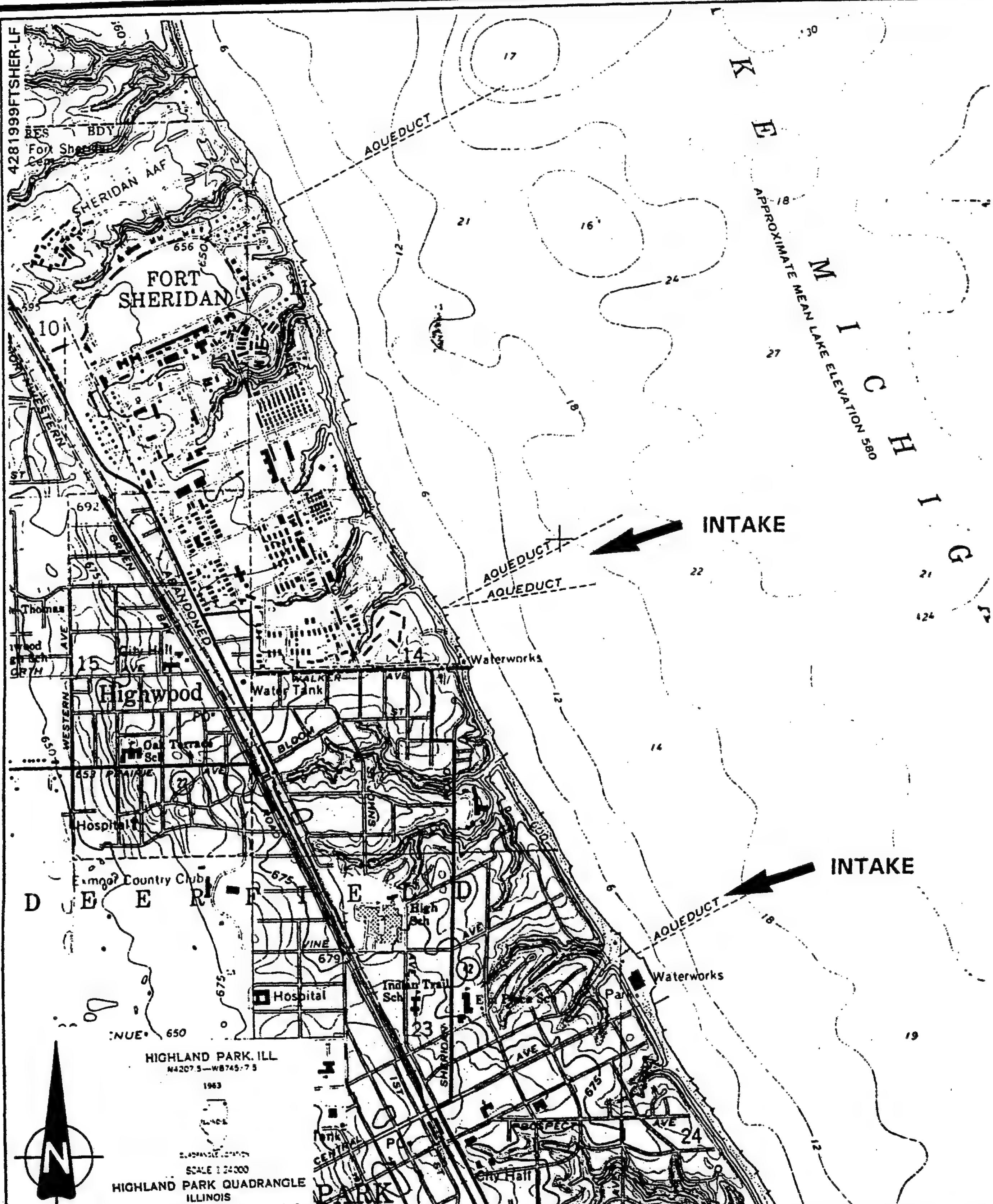

Figure 5-1
LOCATION OF HIGHWOOD AND HIGHLAND PARK WATER INTAKES
Final Anti-Aircraft Artillery Ranges Sampling and Analysis Plan
Fort Sheridan, Illinois

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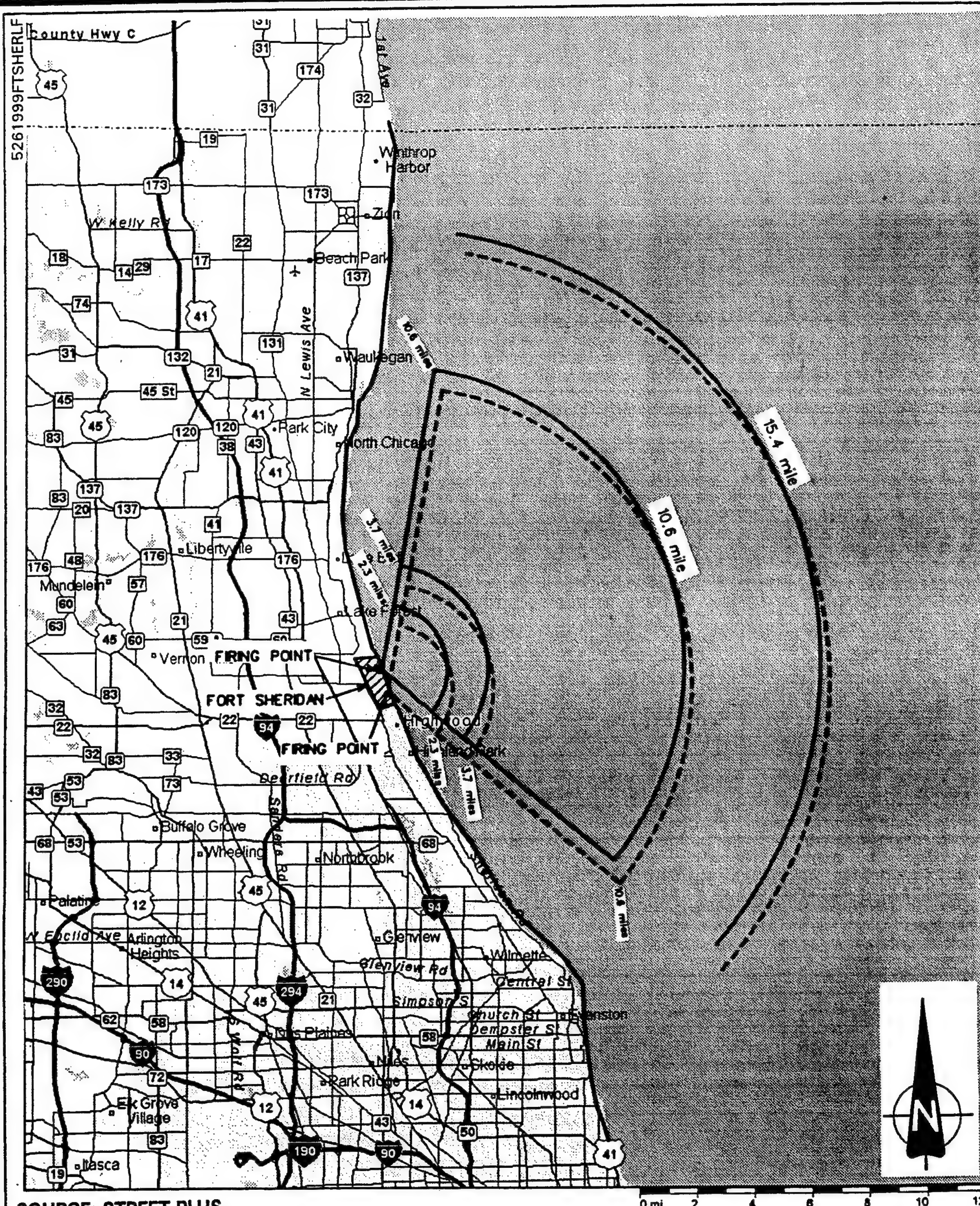

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Figure 5-2
AAA IMPACT ZONES
Final Anti-Aircraft Artillery Ranges Sampling and Analysis Plan
Fort Sheridan, Illinois

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Photographs or a video will be taken at each sampling station and of the area surrounding the intake structure. UXO or potential UXO will not be cleared or removed. Any UXO, potential UXO, or UXO-related scrap encountered will be documented in the field log book and its location determined by GPS. "Live" or "suspect live" UXO will be reported to the Fort Sheridan BRAC office.

5.1.1 Surface Water Sampling

At each sampling station, the surface water samples will be collected directly (approximately 12 inches) above the sediment surface by the scuba diver. Surface water samples will be collected close to the lake bottom because explosives constituents are more likely to be detected in close proximity to the sediment due to dilution in the lake.

The locations of the surface water stations are presented in Figure 5-3. No fewer than eight surface water samples will be collected from each intake. Care will be taken to prevent disturbance of the sediment before water sampling. The surface water sampling will start south of the intakes and proceed northward. Generally, the surface water and sediment flow in this area of Lake Michigan is from north to south. This will prevent the sampling at one station from interfering with the sampling conducted at the upflow stations.

The surface water samples will be collected directly into clean glass jars. The closed jars will be lowered to the sampling point above the sediment and opened. Upon filling, the jars will be resealed and brought to the surface. The jars will be placed in a prechilled ice chest for subsequent shipment to the laboratory.

5.1.2 Sediment Sampling

Upon completion of the surface water sampling at each station, the sediment samples will be collected by the scuba diver (see Figure 5-3). If UXOs are found near the intakes, additional sediment samples will be collected next to the UXO item, without disturbing it. In any event, no fewer than eight sediment samples will be collected from each intake. Based on previous studies indicating that the detection of explosives constituents around UXO is directional, sediment samples will be collected from each of the four compass points (south, east, west, north) around the UXO item. Sediment samples will be collected approximately 6 inches from the UXO item, but no further than 12 inches from the item to maximize the potential for detecting explosives constituents (Darrach and Chutjian, 1997). The sediment samples will consist of the upper sediment (0 to 6 inches). The sediment (if possible) will consist of fine grained (clay-silt-sand) sized material. The larger material (gravel size and above) will be excluded from the sample because the explosives constituents are more likely to be complexed with the fine grained sized material.

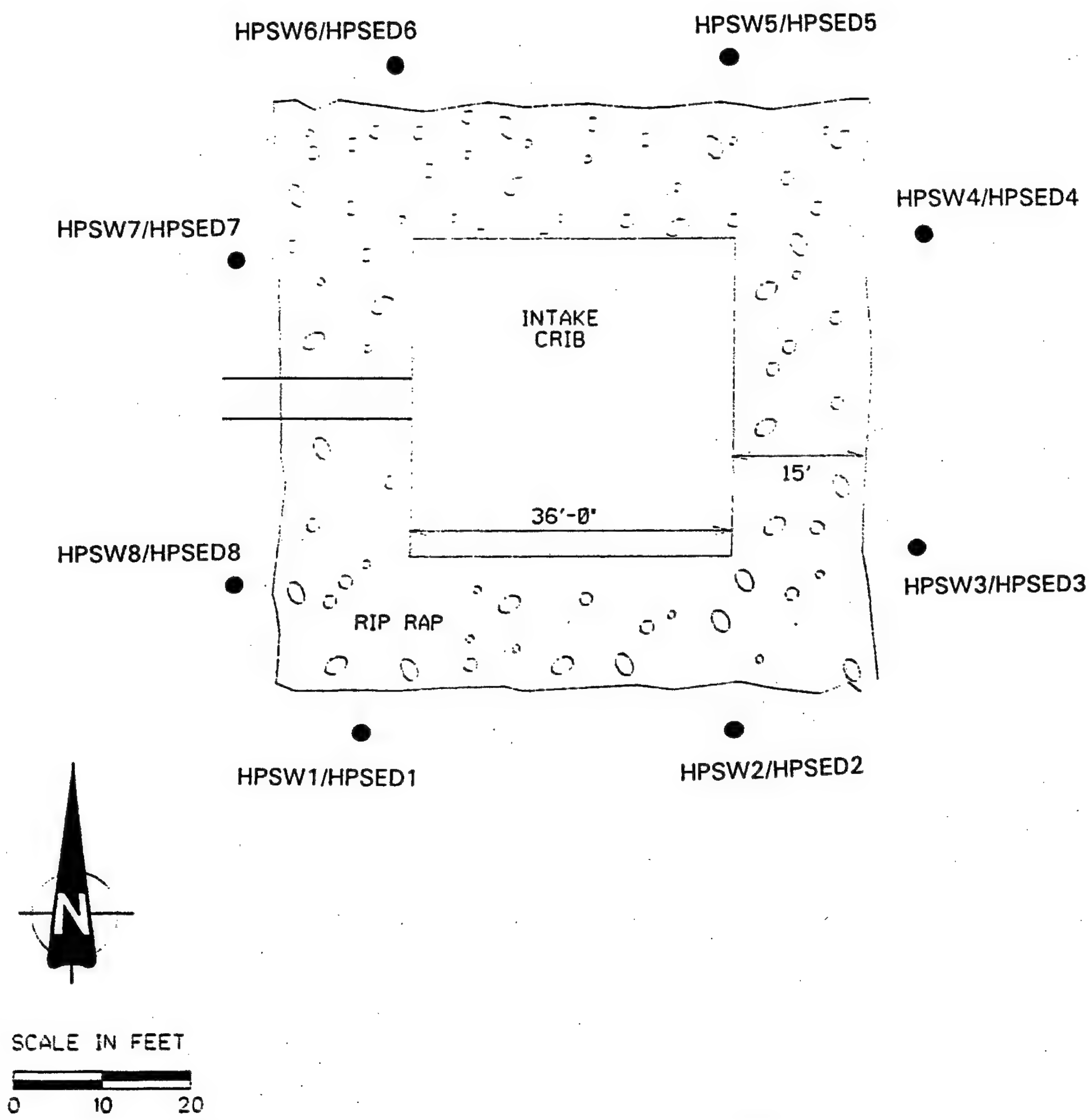

Figure 5-3
INTAKE SURFACE WATER/SEDIMENT SAMPLING STATIONS
Final Anti-Aircraft Artillery Ranges Sampling and Analysis Plan
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Sediment sampling will be conducted by placing the sediment directly into the laboratory glass jars. This will be accomplished (if possible) by directly pushing the jar through the sediment. If necessary, a stainless steel trowel or spoon will be utilized to place the sediment in the jar. When filled, the jar will be resealed and brought to the surface. If difficulty is encountered with obtaining the sediment sampling directly into the sample jars, a petite ponar or Eckman dredge will be used to obtain the samples. These sediment sampling devices will be lowered and retrieved from the boat and guided by the scuba diver, as necessary (e.g., around a UXO item). The ESE SOP for work on or near water involving the use of boats (Appendix F) will be followed during the surface water/sediment sampling. The sediment collected by these devices will be placed in a stainless-steel bowl and mixed before placing in the sample jars. The petite ponar/Eckman dredge sampling SOP is presented in Appendix G.

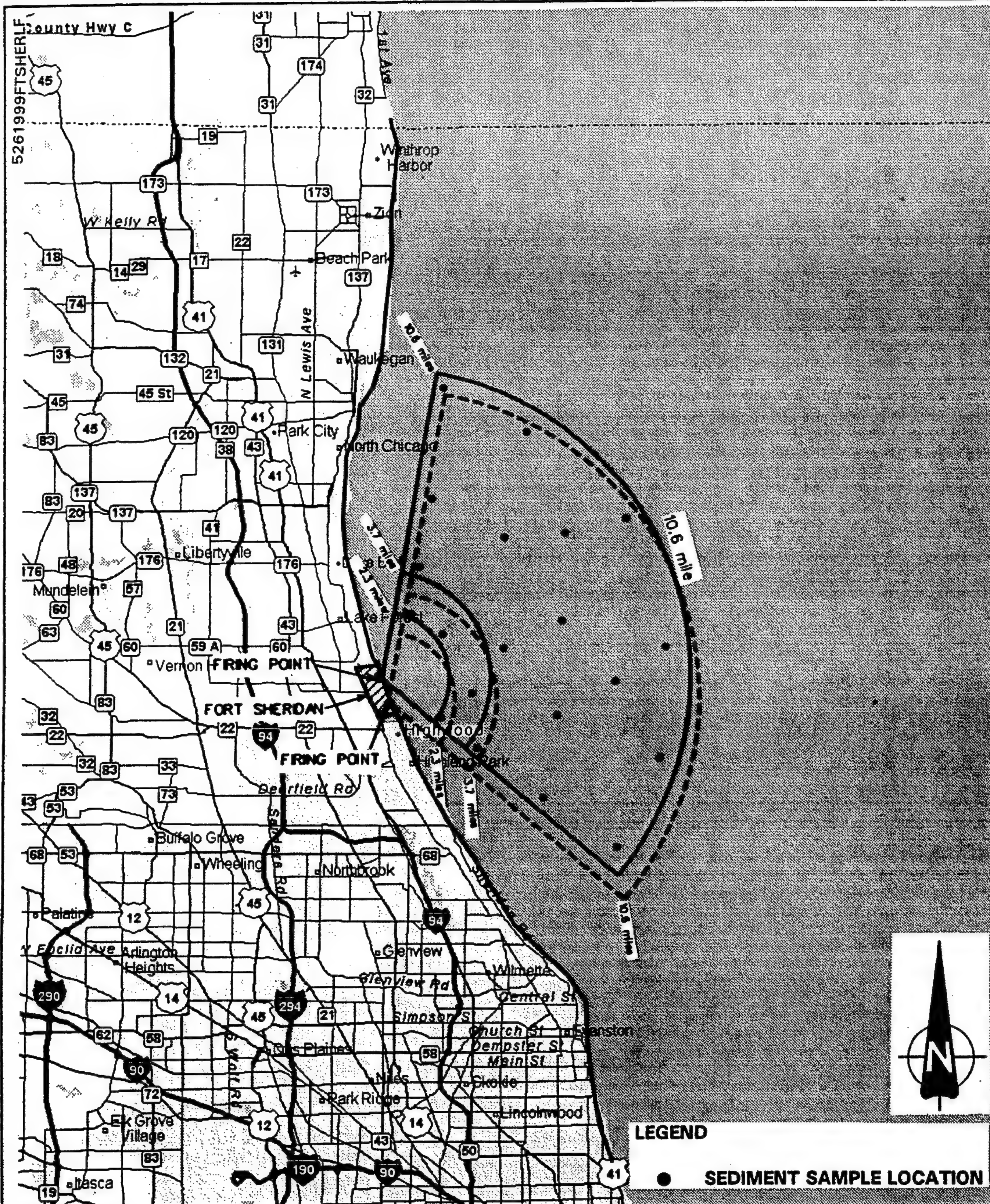
5.2 Water Treatment Plant Sampling

One raw water sample will be collected from the Highland Park and Highwood treatment plants. The raw water samples will be collected directly into a clean glass jar. Every attempt will be made to collect the raw water samples during or after a storm event, in order to get as much suspended sediment in the sample as possible. The filled jar will be placed in a prechilled ice chest for subsequent shipment to the laboratory.

5.3 AAA Impact Zone Sampling

Twenty sediment samples will be collected from the AAA impact zone as indicated in Figure 5-4. This impact zone represents those areas most likely to contain the greatest concentration of UXO based on the firing points and types of artillery used during the training exercises (USACE, 1995). A typical over water firing fan is approximately 120 degrees (Sloan, 1999). As shown in Figure 5-4, 15 samples will be collected in the area expected to contain the greatest concentration and types of UXO (i.e., from 3.7 miles to 10.6 miles from shore) (see Appendix A). Five samples will be collected from the area expected to contain the greatest concentration of lower caliber UXO. The sediment sampling will start in the southern portion of the impact zone and proceed northward.

A Trimble Navigation real-time differential GPS with data logger will be utilized to locate the sediment sampling points. U.S. Coast Guard digital GPS signals will be utilized to provide real-time differential correction of GPS signals. This will increase the GPS location accuracy to ±5 feet. Following the sampling effort, each of the sediment sampling stations will be located on an appropriate scale figure via the GPS coordinates.



SOURCE: STREET PLUS

Figure 5-4
AAA IMPACT ZONE SEDIMENT SAMPLE LOCATIONS
Final Anti-Aircraft Artillery Ranges Sampling and Analysis Plan
Fort Sheridan, Illinois

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A petite ponar or Eckman dredge will be used to obtain the sediment samples. These sediment sampling devices will be lowered and retrieved from a boat. The sediment collected by these devices will be placed in a stainless-steel bowl and mixed before placing in the sample jars.

The sediment (if possible) will consist of fine grained (clay-silt-sand) sized material. The larger material (gravel size and above) will be excluded from the sample. If insufficient fine grained material is present in the sample, two more attempts will be made to obtain sufficient fine grained material. If these subsequent attempts do not yield sufficient fine grained material, the smallest sized material will be placed into the sample jar.

Surface water samples will not be collected along with sediment samples in the impact zone due to the large relative distance between the sediment sampling locations and the municipal water intakes, which are of greatest concern relative to the potential presence of UXO in Lake Michigan. As stated previously, explosive constituents are undetectable at 12 inches or more from an item of UXO and, once dissolved in water, explosive constituents can readily decompose within hours. Conversely, TNT breakdown products form tightly bound complexes with sediments that are resistant to biodegradation (Walsh, 1990; Darrach and Chutjian, 1997; Naval Explosive Ordnance Disposal Technology Center, 1988; Layton *et al*. 1987; Zappi, 1998). The significant dilution factor of Lake Michigan, combined with the large relative distance between the sediment sampling locations and the municipal water intakes and expected fate of explosive compounds in a lake environment, indicate that the collection of surface water samples along with the sediment samples will not provide meaningful data. Sufficient data relative to the potential effects of explosive constituents in Lake Michigan on the municipal water supplies will be provided by the intake and raw water samples.

6.0 Evaluation and Reporting

The data obtained through implementation of this study will be presented in a separate report. The analytical data will be presented in summary tables and recommendations made, as appropriate, regarding the need for additional investigations. Field observations and photographs will also be presented.

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Appendix A

Analysis of AAA Ranges

(Source: USACE, 1995)

Analysis of Ammunition Contamination
in Lake Michigan
due to Anti-Aircraft Artillery Fire
from Fort Sheridan

Anti-Aircraft Artillery training occured at Fort Sheridan from 1930 through the 1950's. The five types of artillery used which had explosive projectiles were the 37mm an 40mm Automatic Guns and the 3in, 90mm, and 120mm (4.7in) guns. If these guns were fired at targets on the surface of Lake Michigan, rounds which did not explode would be found from the shore out to the maximum range of the weapon. However, the vast majority of firing was conducted against towed aerial targets. The following table gives the minimum, maximum, and center of pattern for each weapon. It can be assumed that the greatest number of rounds remaining in Lake Michigan are centered at the center of the pattern and the number decreases as you move away from this center until you reach the minimum and maximum.

AAA RANGES

	Minimum	Maximum	Center of Pattern
37mm Gun	4050 yds 2.3 mi	8875 yds 5.0 mi	6460 yds 3.7 mi
40mm Gun	5000 yds 2.8 mi	10850 yds 6.2 mi	7925 yds 4.5 mi
3in Gun	Not Available	Not Available	Not Available
90mm Gun	8000 yds 4.5 mi	19560 yds 11.1 mi	13780 yds 7.8 mi
120mm Gun (4.7in)	10000 yds 5.7 mi	27160 yds 15.4 mi	18580 yds 10.6 mi

The above data is extracted from Firing Tables, FT 37AA-N-2 (1941), FT 40AA-A-2 (1943), FT 90AA-B-3 (1944), and FT 4.7AA-C-1.

The above information indicates that the majority of unexploded rounds would be from 3.7 miles to 10.6 miles from shore with a decreasing potential of rounds out to 15.4 miles. The depths of Lake Michigan are 45 feet at 3.7 miles, 120 feet at 10.6 miles and 180 feet at 15.4 miles. It must be assumed that a potential exists for unexploded ordnance to extend from the shore line out to the maximum range because of the potential for short rounds and the possibility of firing against a surface target floated on Lake Michigan.

Appendix B

Environmental Fate Information for TNT and Tetryl

AD
UCRL-21109

Conventional Weapons Demilitarization: A Health and Environmental Effects Data-Base Assessment

Explosives and Their Co-Contaminants Final Report, Phase II

D. Layton, B. Mallon, W. Mitchell, L. Hall,
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December 1987

Supported by
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Fort Detrick, Frederick, MD 21701-5012

Project Order 83PP3818

Project Officer: Mitchell J. Small

The findings of this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

2. IDENTIFICATION OF THE PRIMARY CO-CONTAMINANTS ASSOCIATED WITH THE OPEN BURNING AND OPEN DETONATION OF MILITARY EXPLOSIVES

Residues of explosive compounds in soils are often accompanied by co-contaminants consisting of manufacture impurities in the explosives, as well as degradation products resulting from the hydrolysis, photolysis, or biotransformation. The main objective of this section is to identify the co-contaminants that are likely to be found at detectable levels at OB/OD sites. Subsequent sections on the individual explosives and co-contaminants deal with the quantitative aspects of transformation processes (e.g., estimates of degradation half-lives). In order to determine which of the contaminants are of potential importance, we review data on the levels of impurities in explosives, the degradation products resulting from the dominant transformation processes, and the persistence of the various substances in environmental media. Data are derived from laboratory studies dealing with transformation processes and field studies in which soil and water samples were analyzed for the presence of explosive-related residues. Our analyses focus on the six explosives (i.e., TNT, RDX, HMX, tetryl, PETN, and ammonium picrate) shown in Fig. 2-1.

CO-CONTAMINANTS OF TNT

TNT is the most widely used military explosive, and it is also present in the largest quantity in the demilitarization inventory (see Layton et al., 1986). In addition, this explosive has the greatest number of co-contaminants of potential concern. Impurities include 2,4-DNT and 2,6-DNT, DNB, and TNB. Co-contaminants also result from photolytic and microbial degradation processes. TNB is a photolytic product of concern as well as an impurity. The important biotransformation products are the aminodinitrotoluenes. Hydrolysis is not an important source of contaminants.

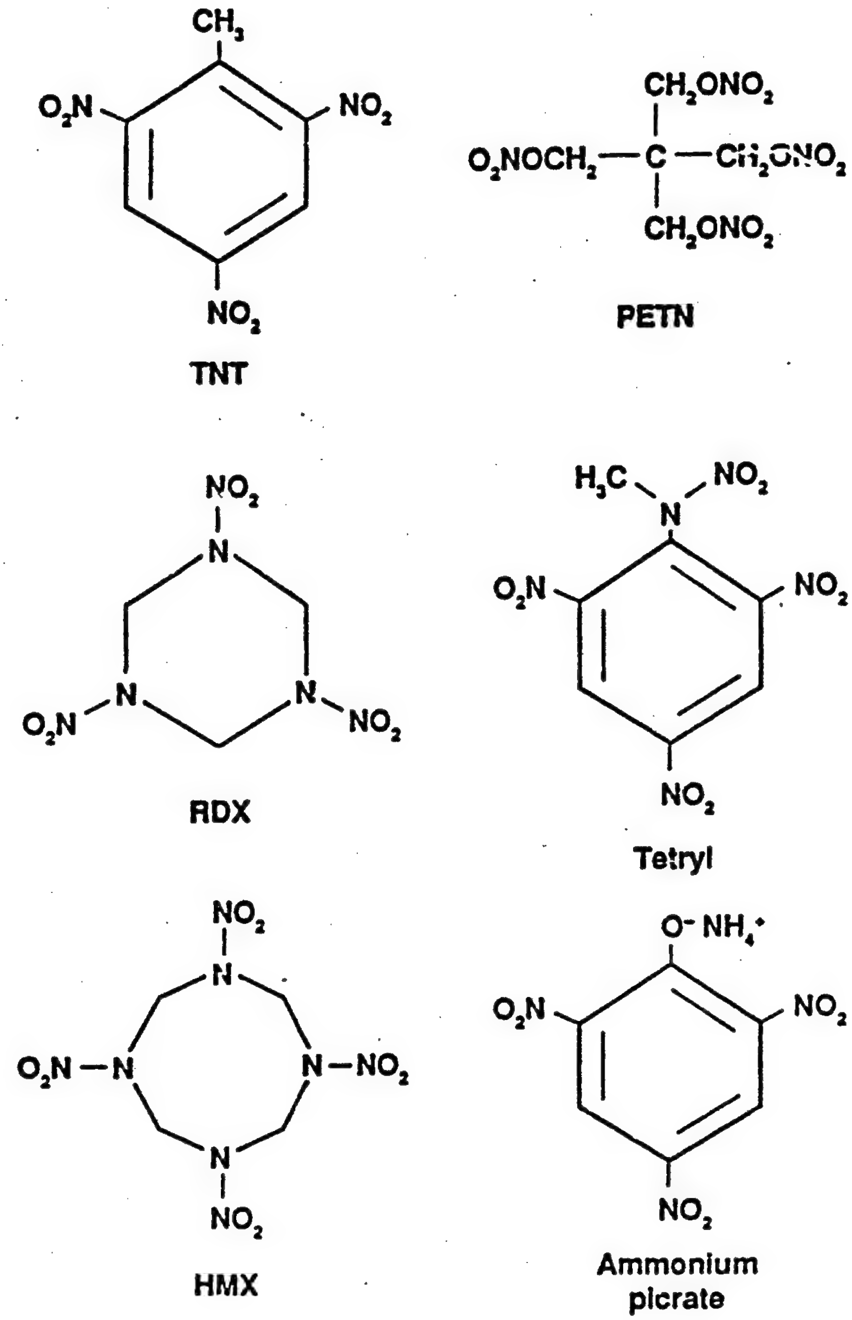


Figure 2-1. Chemical structures of the six explosives addressed in Section 2.

Production and Impurities

TNT is produced in the U.S. by the progressive nitration of toluene in a multistage process that uses mixtures of nitric and sulfuric acid. The primary impurities in TNT result from the incomplete nitration of toluene, oxidation (e.g., methyl-group oxidation), and the production of TNT isomers (see Rosenblatt et al., 1971). Aqueous sodium sulfite solution (sellite) treatment removes the undesirable unsymmetrical TNT isomers by a chemical reaction that converts those isomers to water-soluble salts. The treatment also forms complexes with the preferred isomer. The aqueous extract is called "red water." Impurities reported in military-grade TNT are listed in Table 2-1. Several of the impurities are TNT isomers that were not completely removed by the sellite treatment. The dinitrotoluenes (DNT) listed in Table 2-1 occur because nitration conditions are not sufficient to convert all the DNT to TNT. The benzenes result from either the decarboxylation of benzoic acid derivatives or from nitration of benzene, a contaminant of the toluene feedstock (Spanggord et al., 1982a). Oxidation of the methyl group of TNT accounts for the formation of the alcohol, aldehyde, and acid, while the last four compounds listed in Table 2-1 are condensation products of transient intermediates derived from TNT.

Photolysis Products

The photolytic products of TNT in distilled and natural waters have been studied by several investigators. For example, Burlinson et al. (1973, 1979a) and Kaplan et al. (1975) have studied the photolysis of TNT in distilled water at room temperature with a mercury lamp or the sun as light sources. Table 2-2 lists the photolysis products identified by those researchers. It should be noted that not all of the photolytic products were identified in the above studies. Kaplan et al. (1975), for instance, could not identify about 60 wt% of the products present.

In natural waters and surface soils, transformation of TNT can result from a combination of photolytic and microbial processes. With ample sunlight, photolytic decomposition predominates, but when light is absent, microbial degradation is the sole transformation pathway. Often there is a combination of degradation pathways; for example, TNT can degrade

Table 2-1. Impurities present in military-grade TNT (from Kaye, 1980, p. T244).

Co-contaminant	Maximum wt%
<u>TNT isomers</u>	
2,4,5-TNT	0.30
2,3,4-TNT	0.20
2,3,6-TNT	0.05
2,3,5-TNT	0.05
<u>DNT isomers</u>	
2,6-DNT	0.25
2,4-DNT	0.50
2,3-DNT	0.05
2,5-DNT	0.10
3,4-DNT	0.10
3,5-DNT	0.01
<u>Nitrobenzenes</u>	
DNB	0.02
TNB	0.10
<u>TNT oxidation products</u>	
2,4,6-Trinitrobenzyl alcohol (TNBOH)	0.25
2,4,6-Trinitrobenzaldehyde (TNBAL)	0.20
2,4,6-Trinitrobenzoic acid (TNBA)	0.05
<u>TNT condensation products</u>	
2,2',4,4',6,6'-Hexanitrobibenzyl (HNBB)	0.40
3-Methyl-2,4,4',6,6'-pentanitrodiphenylmethane (MPDM)	0.40
2,2'-Dicarboxy-3,3',5,5'-tetranitro-azoxybenzene (white compound)	0.05
3,3'5,5'-Tetranitroazoxybenzene	0.01

photolytically to trinitrobenzene, which then degrades microbially to nitroanilines, as shown in Fig. 2-2.

Burlinson (1980) placed samples of "raw" Potomac River water spiked with TNT in sunlight, shade, and darkness. A fourth sample of river water was prepared by adding sediment and a nutrient (ethanol). TNT was also added to distilled water. The latter two samples were exposed to sunlight. The degradation products in all the crocks containing the TNT solutions were

Table 2-2. Photochemical degradation products of TNT that have been identified or measured in distilled water.

Product	Light Source: Mercury lamp	Light Source: Sunlight
	References[a]	
TNB	2	1,2,3
TNBOH	2	1,2
TNBAL	2	1,2
TNBA	2	
4,6-Dinitroanthranil	2	
2,4,6-Trinitrobenzonitrile	2	1,2
3,5-Dinitrophenol	2	
2-Amino-4,6-dinitrobenzoic acid	2	
syn-2,4,6-Trinitrobenzaldoxime	2	
N-(2-carboxy-3,5-dinitrophenyl)-2,4,6-trinitrobenzamide	2	3
Four isomers of tetranitroazoxytoluene (see Table 2-3)		1
2,2'-Dicarboxy-3,3',5,5'-tetranitro-azoxybenzene (white compound)	2	
2,2'-Dicarboxy-3,3',5,5'-tetranitro-desoxybenzene (desoxy white)	2	
2-Carboxy-3,3',5,5'-tetranitroazoxy-benzene (monocarboxy white)	2	
1,3,7,9-Tetranitroindazolo-2,1-α-indazol-6-ol-12-one	2	3

[a] 1 = Burlinson et al., 1973.

2 = Kaplan et al., 1975.

3 = Burlinson et al., 1979a (only products at environmentally encountered pH's are listed in Table 2-2)

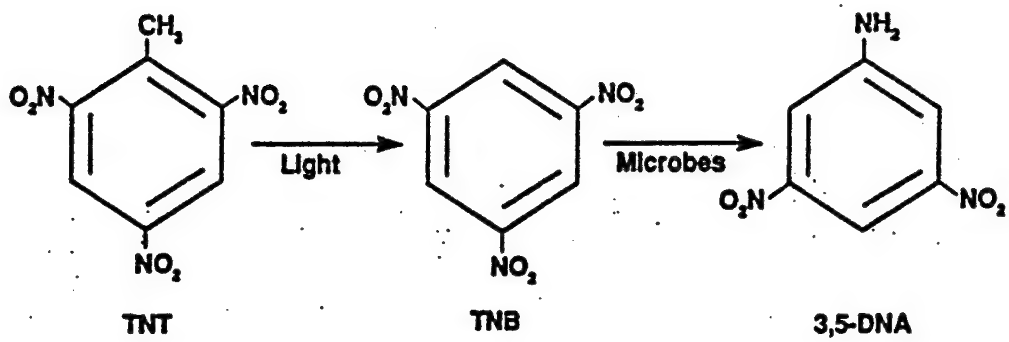


Figure 2-2. An example of photolytic oxidation followed by microbial reduction of TNT.

observed for about 38 d. The substances present in the distilled water that was exposed to sunlight were all photolytic products, such as TNBAL, while the river-water sample kept in the dark produced substances that were all of microbial origin (e.g., aminodinitrotoluenes). The other samples contained a range of products resulting from photolytic (sunlight exposures) and/or microbial (dark) degradation processes. Burlinson proposed that the initial photolytic product is TNBAL, because his distilled water sample exposed to sunlight produced only TNBAL from TNT. In the river-water samples, the TNBAL was quickly converted to TNB, which then degraded microbially to 3,5-dinitroaniline (3,5-DNA). In actual field measurements, Spanggord et al. (1983a) identified many of the same products in analyses of active lagoon waters.

Microbial Products

Many bacteria, fungi, yeasts, and other microorganisms reduce the TNT nitro groups to amines (Spanggord et al. 1980a; McCormick et al., 1976; Parrish, 1977). The biotransformation products that have been identified in various studies are summarized in Table 2-3. Most ^{14}C-ring-labeled-TNT (denoted ^{14}C-TNT) experiments have demonstrated that microbial transformation does not proceed to ring cleavage. One study did, however, report ring

Table 2-3. Biotransformation products of TNT that have been identified by various investigators.

Product	Reference[a]
2-Amino-4,6-dinitrotoluene (2-amino-4,6-DNT)	1,2,3,4,5
4-Amino-2,6-dinitrotoluene (4-amino-2,6-DNT)	1,3,4,5
2,6-Diamino-4-nitrotoluene (2,6-diamino-4-NT)	1,3,5
2,4-Diamino-6-nitrotoluene (2,4-diamino-6-NT)	1,2,3,5
2,4,6-Triaminotoluene (TAT)	2
4-Hydroxylamino-2,6-dinitrotoluene (4-hydroxyamino-2,6-DNT)	2,3
2-Hydroxylamino-4,6-dinitrotoluene (2-hydroxyamino-4,6-DNT)	2
2,2',6,6'-Tetranitro-4,4'-azoxytoluene (4,4'-Azoxy)	1,2,3,5
4,4',6,6'-Tetranitro-2,2'-azoxytoluene (2,2'-Azoxy)	1,2,3,5
4,2',6,6'-Tetranitro-2,4'-azoxytoluene (2,4'-Azoxy)	1,2
2,4',6,6'-Tetranitro-4,2'-azoxytoluene (4,2'-Azoxy)	1,2,5
Product binding with humic acids, lipids, and proteins	6
Nitrate ion	1
Nitrite ion	1

[a] 1 = Kayser et al., 1977.
2 = McCormick et al., 1976.
3 = Spanggord et al., 1980b.
4 = Burlinson, 1980.
5 = Kaplan and Kaplan, 1982a.
6 = Kaplan and Kaplan, 1983.

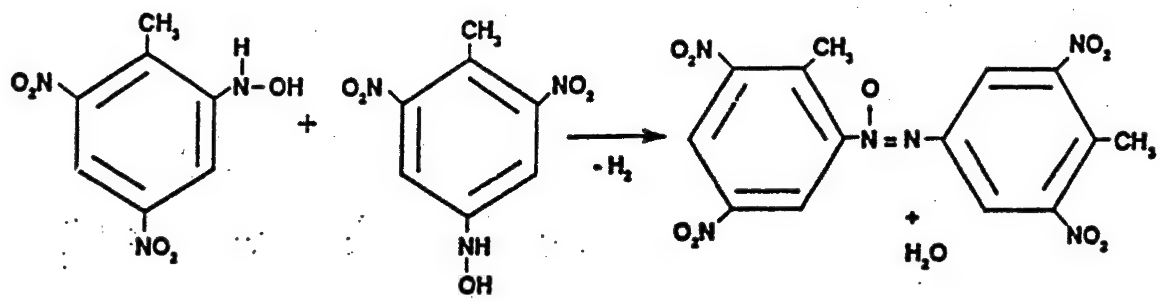


Figure 2-3. Formation of 2,4'-Azoxy from microbial reduction.

cleavage by gram-negative bacteria (Traxler, 1976). In Fig. 2-3 we show dimerization of the reduction intermediates, which produces an azoxy product. The reactive groups can be in either the 2- or the 4- position, making four possible isomers (McCormick et al., 1976).

Microbes reduce TNT faster under aerobic conditions than they do under anaerobic conditions. However, the aerobic products are mostly mono- and diamines, whereas the anaerobic products are mostly di- and triamines. In addition, pH has a strong effect on the extent of the reduction. In a 24-h experiment, Kayser et al. (1977) discovered that TNT was only 45% converted at pH 6-6.2, while it was 99% reduced at pH 7.4-7.8. A high transformation rate continued through pH 9.1; at this point there was an accumulation of amine products. Corn-steep liquor and other nutrients enhance the efficiency of microorganisms in TNT reduction. For some microorganisms, added nutrients are essential for reduction.

Hoffsommer et al. (1978) summarized previous studies conducted at the Naval Ammunition Depot (NAD), McAlester, Oklahoma, that showed that plants grew better in streams contaminated with TNT than in uncontaminated streams. They suggested that several strains of bacteria biotransform TNT into aminonitrotoluenes, thus supplying additional nutrients for plant life. The TNT-reduction products, 2-amino-4,6-DNT and 4-amino-2,6-DNT, were detected in TNT-contaminated streams at NAD, McAlester, Oklahoma. In addition, three species of microorganisms (pseudomonads) that were especially adapted for TNT reduction were isolated from the waters.

Plant and Human Metabolites

Palazzo and Leggett (1986) studied the effect of TNT on yellow nutsedge (Cyperus esculentus) grown in hydroponic aquaria. After 42 d in 5 to 20 mg/L solutions, the nutsedge had absorbed TNT and produced two metabolites: 2-amino-4,6-DNT and 4-amino-2,6-DNT. These metabolites were present in the plant roots at up to 18 times the concentration of TNT. The tubers also showed the same pattern of absorption and metabolism, but to a smaller extent. Other products were not identified.

Metabolism in humans produces most of the same products as photolysis and microbial degradation: the methyl group is progressively oxidized, and the nitro group is reduced. Azoxy compounds result from coupling. Yinon and Hwang (1985) list the following metabolic products of TNT: 2-amino-4,6-DNT, 4-amino-2,6-DNT, 4-hydroxyamino-2,6-DNT, 2,4-diamino-6-NT, 2,6-diamino-4-NT, TNBA, TNBOH, 4,4'-azoxy, and 2,2'-azoxy.

Hydrolysis Products

TNT reacts with bases, especially at high temperature (Kaye, 1980, pp. T251-T253). However, no hydrolysis of TNT was observed at a pH of approximately 8, after 108 d in seawater (Hoffsommer and Rosen, 1973). Therefore we do not expect hydrolysis to be a significant source of degradation products.

Complex and Compound Formation

Less TNT and its biotransformation products can be extracted from organic-rich soils and sediments than would be expected on the basis of calculated and measured partition coefficients (Kaplan and Kaplan, 1982b; Hoffsommer et al., 1978; Carpenter et al., 1978; Isbister et al., 1984; and Spanggord et al., 1980b). McCormick (1986) suggested that microbial reduction of TNT produces highly substituted anilines or their precursors, which react immediately with carboxyl groups of humic acids, lipids, or proteins to form insoluble precipitates. In research that supports this hypothesis, Kaplan and Kaplan (1983) performed tests with humic acids, and observed binding with TNT and the two amino reduction products, 2,4-diaminonitrotoluene and

2,6-diaminonitrotoluene. They concluded that TNT itself did not bind to the humic acids, but the two amines bound readily. The binding reactions were enhanced at increased acidity (pH 4), where the amines were more reactive. The fate or toxicity of the complexes is unknown. Similarly, Spanggord et al. (1980b) recovered much less than the expected amount of TNT from unsterilized sediment when measuring sediment/water partition coefficients. They suggested that either biodegradation, irreversible sorption, or reactions with the sediment may account for the unrecovered TNT.

In the above experiments, TNT probably remained in organic matter and in the bacterial floc at higher levels than those expected on the basis of the low soil/water partition coefficient measured in the sterilized soil. Other factors (e.g., chemical reactions, complex formation, etc.) need to be studied to define the extent of the interactions of TNT and its degradation products, with naturally occurring organic substances.

Field Measurements of TNT and Its Degradation Products

The results of field measurements of TNT and associated compounds in soils and ground and surface waters are consistent with the above laboratory studies. Spanggord et al. (1983a) studied the disposition of TNT and RDX in waste disposal lagoon waters at the Louisiana Army Ammunition Plant. They found a combination of photolytic transformation products and biotransformation products similar to those found by Burlinson (1980) in experiments with Potomac River water. The products were TNB; 3,5-DNA; the amino-DNT isomers; TNBOH; 3,5-dinitrophenol; 2-hydroxy-4,6-dinitrobenzoic acid; 2-amino-4,6-dinitrobenzoic acid; and 2,4-dinitrobenzoic acid. The major product was 2-amino-4,6-dinitrobenzoic acid.

Some of these products appear repeatedly in analyses of samples of soil and natural waters. Mizell et al. (1982) reported concentrations of TNT, 2,4-DNT, 2,6-DNT, DNB, and TNB in ground and surface waters and soils associated with former TNT washout lagoons and open burning/open detonation sites at the Blue Grass facility in Kentucky. Many other investigators (e.g., Rosenblatt, 1981; Newell, 1984; Swisdak and Young, 1977) report concentrations of TNT and the DNT's in soils and in ground water. Co-contaminants of TNT in ground water were TNB (Rosenblatt, 1981), 2,4-DNT, 2-amino-4,6-DNT and

4-amino-2,6-DNT (Swisdak and Young, 1977). The latter three compounds were in shallow ground water, beneath and downgradient from a TNT disposal site.

The above information indicates a gradual movement through soils and ground water of TNT, its photolysis product, TNB; impurities--the DNT's; and its biodegradation products, aminonitrotoluenes and aminotoluene. The other compounds that may have been formed have not been reported in the literature.

Summary

The large array of potential TNT co-contaminants and conversion products that we have identified are presented in Table 2-4. The compounds are arranged by functional groups: nitroaromatic isomers of TNT and DNT; methyl-group oxidation products; nitro-group reduction products; and dimers of some of the above compounds. The most prevalent of the nitroaromatic impurities from the manufacturing process are 2,4-DNT and 2,6-DNT. The other compounds are impurities that were incompletely removed by the sellite treatment.

The co-contaminant TNB is both a manufacturing impurity and the result of photolytic oxidation and decarboxylation of the methyl group of TNT. Likewise, TNBOH, TNBAL, and TNBA are the result of photolytic oxidation of the same methyl group, but to a lesser extent. The anthranils, nitriles, and oxime in Table 2-4 are also of photolytic origin, possibly by a different mechanism. Finally, the degradation products detected in lagoons, 3,5-dinitrophenol and 2-amino-4,6-dinitrobenzoic acid, are a combination of photooxidation of the methyl group and reduction and/or substitution of the nitro group. Amines result from the metabolic reduction of the nitrotoluenes.

The remaining compounds in Table 2-4 are condensation products of the compounds listed previously. They result from all sources: impurities, photolytic products, and microbial products. They may represent final products or they too may condense or complex with other TNT transformation products or humic materials, proteins, and other natural compounds. The compounds in Table 2-4 that have been detected in the environment were isolated from field samples. Excluded from the review were substances from sources such as holding ponds, lagoons, and waste-water discharges. In our

Table 2-4. Co-contaminants and degradation products of TNT in the environment.

Source	Compound	Detected in environment?[a]
Parent	TNT	Yes
Impurity	2,4,5-Trinitrotoluene	
Impurity	2,3,4-Trinitrotoluene	
Impurity	2,3,6-Trinitrotoluene	
Impurity	2,3,5-Trinitrotoluene	
Impurity	2,4-DNT	Yes
Impurity	2,6-DNT	Yes
Impurity	2,3-DNT	
Impurity	2,5-DNT	
Impurity	3,4-DNT	
Impurity	3,5-DNT	
Impurity Photolysis	TNB	Yes
Impurity Photolysis	DNB	Yes
Impurity Photolysis Thermal	2,4,6-TNBOH	
Impurity Photolysis Thermal	TNBAL	
Impurity Photolysis Metabolism	TNBA	
Metabolism Photolysis	3,5-Dinitrophenol	
Photolysis Thermal	4,6-Dinitroanthranil	
Photolysis	2,4,6-Trinitrobenzonitrile	

Table 2-4. (Continued)

Source	Compound	Detected in environment?[a]
Photolysis	syn-2,4,6-Trinitrobenzaldoxime	
Photolysis	2-Amino-4,6-dinitrobenzoic acid	
Microbial	4-Hydroxylamino-2,6-DNT	
Microbial	2-Hydroxylamino-4,6-DNT	
Microbial	2-Amino-4,6-DNT	Yes
Microbial	4-Amino-2,6-DNT	Yes
Microbial	2,4-Diamino-6-NT	
Microbial	2,6-Diamino-4-NT	
Microbial	2,4,6-Triaminotoluene	
Microbial	3,5-DNA (from TNB)	
Impurity	HNBB	
Impurity	MPDM	
Impurity Photolysis	White compound	
Impurity	3,3',5,5'-Tetranitroazoxybenzene	
Photolysis Microbial	4,4'-Azoxy 2,2'-Azoxy 2,4'-Azoxy 4,2'-Azoxy (See Table 2-3)	

Table 2-4. (Continued)

Source	Compound	Detected in environment?[a]
Photolysis	Desoxy white compound	
Photolysis	Monocarboxy white	
Photolysis	N-(2-Carboxy-3,5-dinitrophenyl)-2,4,6-trinitrobenzamide	
Photolysis	1,3,7,9-Tetranitroindazolo-2,1-α-indazol-6-ol-12-one	
Microbial	Nitrate ion	
Microbial	Nitrite ion	

[a] Compounds with a "yes" appearing after them have been measured in environmental media; the other compounds in this table may also occur, but have not been reported.

data-base assessments we will examine DNB, TNB, 2,4-DNT, 2,6-DNT, and the two aminodinitrotoluenes, as these co-contaminants have been frequently detected in environmental media.

Although humic or plant-tissue complexes with TNT degradation products have not been isolated from field samples, laboratory evidence for them is strong. Little is known of their structures, fates, or toxicity. Laboratory experiments also show that plant tissues accumulate TNT and, more extensively, the 2-amino-4,6-DNT and 4-amino-2,6-DNT metabolites. It is not known if these compounds are free or are bound in some way. We consider the complexes of TNT, 2-amino-4,6-DNT and 4-amino-2,6-DNT with humic and plant materials of secondary concern from an environmental standpoint, only because they have not been found in the field. Clearly, more research needs to be conducted in this area.

CO-CONTAMINANTS OF RDX

The second most important explosive in the demilitarization inventory is RDX. Large-scale usage of RDX began during WW II. One common explosive

one instance did aerobic microbial degradation occur--in Holston River water or effluent from the Holston Army Ammunition Plant to which yeast was added.

Hydrolysis Products

Little research has been done on the hydrolysis of HMX. Croce and Okamoto (1979) measured an alkaline hydrolysis rate constant for HMX that was an order of magnitude lower than for RDX. No mention was made of hydrolysis products, but they are probably analogous to those of RDX; at pH 13, RDX was hydrolyzed to gaseous products and formate ion (Hoffsommer *et al*., 1977).

Field Measurements of HMX Contamination

Newell (1984) reported the concentration of HMX in soils at open-burning sites at depths of 15 to 45 cm to be slightly higher than at the surface or in "residue" samples at the same site.

Summary

Photolysis is the major degradation route for HMX in flowing streams and well-lighted waters. During photolytic degradation, HMX is transformed to low-molecular-weight compounds. Photolysis of solid HMX results in the formation of some polymeric products, probably due to the confinement of the intermediates. Only with added nutrients and anaerobic conditions will HMX degrade microbially. Rarely does aerobic decomposition take place. Table 2-6 lists the co-contaminants of HMX discussed in this section.

CO-CONTAMINANTS OF TETRYL

Tetryl has been used as a booster explosive and as a main charge in the form of a mixture with TNT (e.g., tetrytol). Tetryl is structurally similar to TNT, with a methylnitramine group replacing the methyl group of TNT. This substitution facilitates the hydrolysis of tetryl to picrate ion and methylnitramine. Photolysis produces N-methylpicramide. Tetryl has a stable aromatic ring that remains intact during environmental transformation processes.

Table 2-6. Co-contaminants and degradation products of HMX.

Source	Compound	Detected in environment?[a]
Parent	HMX	Yes
Impurity	RDX	Yes
Photolysis Thermal	Nitrogen	
Photolysis Thermal	Nitrous oxide	
Photolysis	Nitrite ion	
Photolysis	Nitrate ion	
Thermal	Hydroxymethylformamide	
Microbial	1,1-Dimethylhydrazine	
Photolysis	Formaldehyde	
Photolysis Thermal	Carbon dioxide	
Photolysis	Ethyne	
Photolysis	Ethene	
Microbial	1-Nitroso-3,5,7-trinitro-1,3,5,7-tetraazocine	
Microbial	1,3-Dinitroso-5,7-dinitro-1,3,5,7-tetraazocine	
Microbial	1,3,5-Trinitroso-7-nitro-1,3,5,7-tetraazocine	
Microbial	1,5-Dinitroso-3,7-dinitro-1,3,5,7-tetraazocine	
Microbial	1,3,5,7-Tetranitroso-1,3,5,7-tetraazocine	

a Compounds with a "yes" after them have been measured in environmental media; the other compounds may also occur, but have not been reported.

Production and Impurities

Tetryl has been produced by two processes. One process involves the addition of N,N-dimethylaniline in concentrated sulfuric acid to fuming nitric acid. One methyl group of N,N-dimethylaniline is oxidized to volatile CO_2 as the aromatic ring and amine of the compound are progressively nitrated. The other method consists of forming dinitromethylaniline from dinitrochlorobenzene and monomethylamine. The dinitromethylaniline is subsequently dissolved in sulfuric acid, and the addition of nitric acid forms tetryl (Urbanski, 1986b).

Tetryl from the above processes is subsequently purified to remove residual acid and byproducts such as compounds with an additional nitro group on the aromatic ring (Urbanski, 1986b). These impurities would have adverse effects on the storage stability of tetryl. The explosive can be used with a variety of epoxies, rubbers, silicones, and adhesives. It is compatible with aluminum, tin, copper, nickel, lead, titanium, silver, cadmium, bronze, and copper-plated steel. It reacts slightly with steel, zinc, and zinc-plated steel, and is incompatible with iron (Department of the Army, 1967b).

On the basis of vacuum stability tests, Farey and Wilson (1975) infer that tetryl can be stored at "normal" temperatures for many years without detectable decomposition. Tetryl's stability is due to the lack of impurities that might cause lower-melting-point eutectics and thus chemical decomposition. Furthermore, its melting point (i.e., 130 to 132°C) is well above stockpile temperatures. When heated alone for 4 wk at 80°C, tetryl degrades to 0.42% N-methylpicramide and 0.16% picric acid. Under the same conditions with lead azide, N-methylpicramide production increased to 2.41%; picric acid to 1.32%; and additionally, picramide, 6.70%, and 4-nitroaniline, 0.47%, were produced. At 120°C, Dubovitskii _et al_. (1961) isolated thermal decomposition products consisting of 2,4,6-trinitroanisole, picric acid, N-methyl-picramide, CO_2, CO, NO, NO_2, and N_2. They also found that picric acid accelerates the decomposition.

Photolysis Products

Kayser _et al_. (1984) reported that N-methylpicramide was the major detectable photoproduct of tetryl in distilled water exposed to laboratory

"room light." They stated that the photolysis rate is at least an order of magnitude greater than the hydrolysis rate.

Microbial Products

We could find no data on the microbial transformation of tetryl. However, the work of McCormick et al. (1976) showing reduction of many aromatic nitrocompounds to their amine counterparts, suggests, by analogy, that the nitro groups of tetryl may also be reduced to amines. Another microbial interaction might be the reduction of hydrolysis or photolysis products, for example, picric acid to picramic acid.

Hydrolysis Products

Kayser et al. (1984) measured the hydrolysis of tetryl both in laboratory light and in the dark. A solution of 12 mg/L of tetryl in distilled water produced different products at different rates depending on light. Because the rate was an order of magnitude slower in the dark, a borax buffer was added and the pH adjusted to 9. In the buffered solution in the dark, tetryl hydrolyzed to methylnitramine as the primary product (66%), picrate ion (28%); NO_2^- (4.1%), NO_3^- (3.1%) and N-methylpicramide (4.1%). On the other hand, the distilled water in laboratory room light hydrolyzed or photolyzed to N-methylpicramide (41.0%) as the major product. These investigators also report increasing hydrolysis rates with both pH and temperature. Urbanski (1986b) states that tetryl reacts with weak bases and strong acids, but not with dilute mineral acids. When tetryl is boiled with dilute alkali (or concentrated acid), the nitroamino group is hydrolyzed.

Complex and Compound Formation

Kayser and Burlinson (1982) analyzed water samples derived from a soil-leaching experiment conducted by Hale et al. (1979). Leachates had passed through 15 cm of soil samples spiked with ^{14}C-labeled tetryl. After 6 mo of irrigation, they found 5 to 14% of the radioactivity in the leachate to be picric acid. Further extraction studies showed many water-soluble and polar products, but no volatile products or tetryl. Lakings and Gan (1981)

found that tetryl was very difficult to recover from plant and animal tissue. They concluded that it was "adsorbed" by the protein or other macromolecules in the tissue matrices. The amine in tetryl or a reduced nitro group apparently reacts in a fashion similar to the amino reduction products of TNT, and, like them, may bind with humic acids, proteins, and lipids.

Field Measurements of Tetryl Contamination

The study by Newell (1984) of the explosives' contamination of soils at OB/OD sites showed that tetryl was present at concentrations as high as 1000 μg/g at several sites.

Summary

Existing studies seem to indicate that the co-contaminants of tetryl (see Table 2-7) will have aromatic structures. Unlike TNT, tetryl undergoes hydrolysis in varying degrees, yielding picric acid or picrate ion and methylnitramine. Photolysis of tetryl produces N-methylpicramide as the major product. This compound has not been identified in environmental media. Tetryl and its amine biotransformation products are likely to complex with humic acids, lipids, and proteins, based on the difficulty in extracting tetryl from soils, plants and animal tissue. Because picric acid is formed by tetryl transformation and occurs as ammonium picrate (Explosive D), it as a compound of primary concern. Methylnitramine and N-methylpicramide are of secondary concern because they have not been found in environmental media.

CO-CONTAMINANTS OF PETN

PETN is a nonaromatic explosive whose production began after WW I. It has been used extensively as a mixture with TNT in small caliber projectiles and grenades. To a lesser extent, it has been used in detonating fuses, boosters, and detonators (Department of the Army, 1967b). Recently, PETN has been incorporated in a rubber-like matrix ("sheet explosive") and used for forming, cladding and hardening metals (Kaye, 1978). It is also used medically as a drug in the treatment of high blood pressure.

Table 2-7. Co-contaminants and degradation products of tetryl.

Source	Compound	Detected in environment?[a]
Parent	Tetryl	Yes
Co-contaminant	TNT	
Thermal	Nitric oxide	
Thermal	Nitrogen dioxide	
Hydrolysis	Nitrate ion	
Hydrolysis	Nitrite ion	
Thermal	Nitrogen	
Thermal	Carbon monoxide	
Thermal	Carbon dioxide	
Hydrolysis	Methylnitramine	
Thermal	Picramide	
Photolysis Hydrolysis Thermal	N-Methylpicramide	
Thermal	4-Nitroaniline	
Thermal	2,4,6-Trinitroanisole	
Thermal	PiOH	
Hydrolysis	Picrate ion	

[a] Tetryl has been measured in environmental media, the other potential co-contaminants may also occur, but have not been reported.

Photolysis

Previous research has shown that the rate of TNT photolysis depends in part on the concentration of photoproducts. Spanggord et al. (1980b), for example, showed that photolysis of a 0.11-mg/L aqueous solution of TNT resulted in disappearance of the dissolved TNT at a linear rate for approximately 100 minutes; then concentrations decreased at a faster rate. In contrast, photolysis of 0.022 mg/L TNT resulted in linear disappearance after 350 minutes. Interestingly, because of the rate acceleration induced by decomposition products, there was less TNT remaining in the more concentrated solution at 250 minutes than in the less concentrated one. Mabey et al. (1983), referring to the above study, noted that the photolysis rate after 33% TNT loss from the initial 0.11-mg/L solution above was 20 times greater than the initial rate. Spanggord et al. (1980b) examined the photolytic degradation of 1.1 mg/L TNT in samples of natural waters exposed to sunlight in borosilicate tubes. Photolysis rate constants were $120 \times 10^{-5}\ s^{-1}$ for Holston River water, $15 \times 10^{-5}\ s^{-1}$ for Waconda Bay water, and $58 \times 10^{-5}\ s^{-1}$ for Searsville Pond water. Photolysis of TNT in distilled water, by comparison, proceeded with a rate constant of $1.7 \times 10^{-5}\ s^{-1}$. In other work, Spanggord et al. (1983a) estimated the half-life of TNT in lagoon water from the Louisiana Army Ammunition Plant at a depth of 50 cm to be 8 d in the middle of March, based on a depth-independent rate constant of 4.3 cm/d. Mabey et al. (1983) estimated the photolytic half-life of TNT in pure water at 40°N latitude using the calculational procedure of Zepp and Cline (1977). In summer the half-life was estimated to be 14 h and in winter, 45 h, based on a quantum yield of 2.7×10^{-3} and absorption coefficient data for TNT.

Mabey et al. (1981) completed two experiments to study the enhancement of photolysis rates by humic substances in natural waters. In the first experiment, aqueous samples (filtered through a 0.2-µm filter) contained in borosilicate reaction tubes were illuminated by single wavelengths from a mercury lamp; in the second experiment, samples in either the borosilicate tubes or in dishes were illuminated by sunlight (May to June in Menlo Park, Ca.). The photolysis half-lives corresponding to the rate constants obtained from the experiments conducted with a mercury lamp (see Table 3-5) and natural waters ranged from about an hour to 5.9 h. The TNT in a pure-water sample, in contrast, had a half-life of about 27 h. When sunlight was used, the photolysis rates of TNT in pure water were also slower than the rates for the

natural waters. In addition, they found that the geometry of the solution vessel influenced the rates of photolysis. For example, a tube receives sunlight from more directions than a dish and consequently, photolytic rate constants for tube experiments were faster than the dish equivalent. Mabey et al. (1981) suggested that photolysis of TNT in natural waters proceeds through an excited triplet state of TNT and that acetone or humic materials act as sensitizers for the production of the excited state, which hastens photolysis. This is supported by the fact that TNT degrades rapidly in natural, sunlit waters in spite of the fact that TNT does not absorb light wavelengths above 400 nm (Spanggord, 1980b). Mabey et al. (1981) noted that the ten-fold difference in photolysis rate constants for pure and natural waters is consistent with photolytic enhancement by humic substances. Additional confirmation of the action of humic acids as triplet sensitizers was provided by comparing the rate constants and quantum yields of TNT photolysis with humic acids present with rate constants of a known triplet sensitizer (see Mabey et al., 1983). On the other hand, Burlinson (1980) found that the rates of disappearance of TNT dissolved in Potomac River water and in distilled water (samples were in dishes exposed to sunlight) were not greatly different.

Photolysis rates are also dependent on pH, according to studies by Burlinson et al. (1979a, 1979b). Burlinson et al. (1979b) proposed that in the photolytic degradation of TNT, one of the adjacent nitro group attracts a hydrogen from the methyl group (I) (see Fig. 3-1), which then transfers the H^+ to form the 2,4,6-trinitrobenzyl anion (II). The course of the reaction depends on the pH of the solution; that is, with an excess of protons (acid), the ion (II) is returned to TNT and with an absence of protons (base), the ion (II) is oxidized to an alcohol, aldehyde, or carboxylic acid, or converted to a nitrile. Some of these products react further or combine to form larger molecules. The carboxylic acid can decarboxylate to form TNB.

Burlinson et al. (1979a) measured the disappearance of TNT in aqueous solutions under varying pH's and found that the half-life of disappearance was inversely proportional to pH. A mercury lamp was used with a Pyrex filter to screen out UV light with wavelengths shorter than 280 nm. At pH 7.4 the half-life of TNT was about an hour, but at pH 3.6 the half-life was about 2 h. Mabey et al. (1983) measured the photolysis rate of 1.1 mg/L TNT in

Table 3-5. Summary of TNT photolysis rates derived from experiments conducted by Mabey et al. (1981).

Light source[a]	TNT concentration (mg/L)	Water sample	Reaction vessel	Rate constant for photolysis ($\times 10^5$ s^{-1})
313 nm[b]	0.63	Holston R., South Fork	Tube	6.29
313 nm[b]	0.63	Searsville Pond	Tube	10.4
366 nm[c]	0.63	Searsville Pond	Tube	18.8
313 nm[b]	0.63	Searsville Pond[d]	Tube	3.27
313 nm[e]	0.28	Searsville Pond[d]	Tube	9.66
313 nm[e]	0.57	Searsville Pond[d]	Tube	8.32
313 nm[e]	1.01	Searsville Pond[d]	Tube	7.16
[illegible] nm[b]	0.63	Pure water	Tube	0.71
Sunlight[f]	Unspecified	Holston R., North Fork	Tube	49.7
Sunlight[f]	Unspecified	Holston R., South Fork	Tube	48.8
Sunlight[f]	Unspecified	Pure water	Tube	4.62
Sunlight[g]	Unspecified	Holston R., South Fork	Tube	103
Sunlight[g]	Unspecified	Holston R., South Fork	Dish	29.7
Sunlight[g]	Unspecified	Holston R. with sediment	Dish	20.9
Sunlight[g]	Unspecified	Searsville Pond	Tube	167
Sunlight[g]	Unspecified	Searsville Pond	Dish	50
Sunlight[g]	Unspecified	Searsville Pond	Flask	84
Sunlight[g]	Unspecified	Pure water	Tube	19

a Mercury lamps were used for the single-wavelength light sources.

b Light intensity of 1.37×10^{-6} einstein^{-1} cm-s.

c Light intensity of 9.85×10^{-6} einstein^{-1} cm-s.

d One-to-one dilution with pure water.

e Light intensity of 7.67×10^{-7} einstein^{-1} cm-s.

f Afternoon sun in May, Menlo Park, Ca.

g Midday sun in July, Menlo Park, Ca.

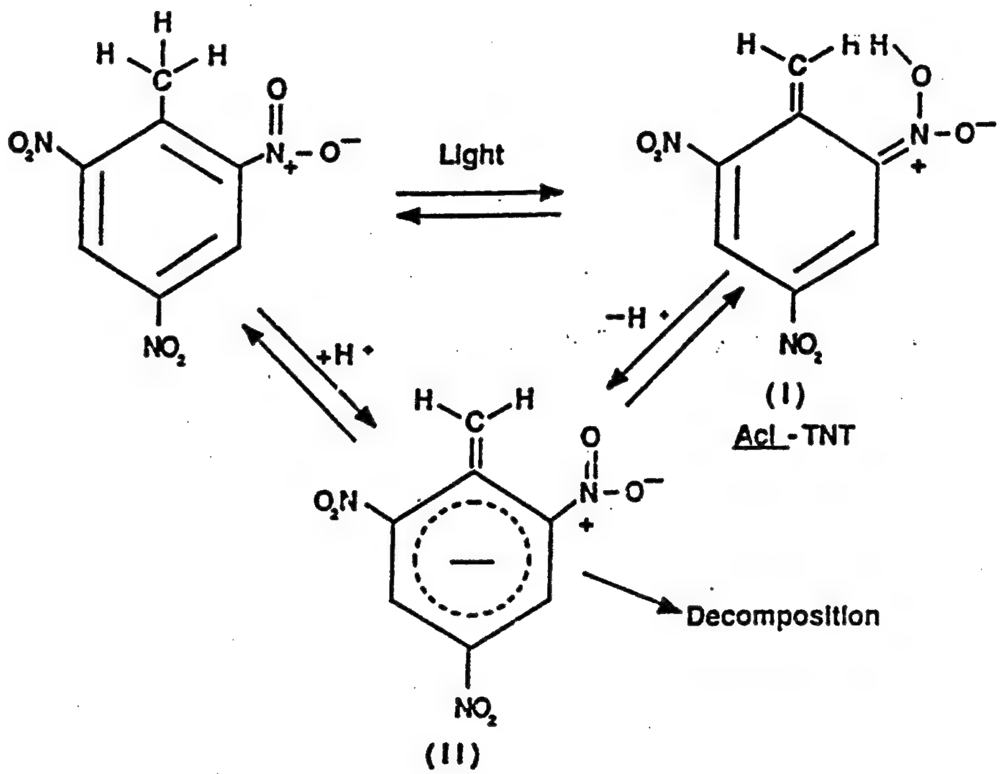


Figure 3-1. Proposed photolytic degradation mechanisms (Burlinson et al., 1979b).

humic-rich Searsville Pond water and pure water at pH 4 and 8. They found only a small effect of pH on TNT photolysis in natural waters; however, in pure water the photolysis half-lives were 12 and 3.7 h, respectively, for pH 4 and 8.

Burlinson et al. (1979a) also showed that the composition of photoproducts varies with pH. For example, between pH 3.6 and 7.4 the major products were 2-amino-4,6-dinitrobenzoic acid, TNBAL, and 4,6-dinitroanthranil. At pH 8.0 to 8.5, the major photoproducts were TNB, TNBAL, and a trace of TNBOH. At pH 11.1 only TNB was present. Also, the photoproducts were the same regardless of whether sunlight or a mercury lamp was used.

The TNT photolysis products listed above are those isolated from irradiated solutions and are not necessarily the final products. Some products of TNT photolysis are also photo-sensitive. Kaplan et al. (1975) and Burlinson et al. (1979a) studied the reactions and products resulting from photolysis of TNBAL, TNBOH, and 4,6-dinitroanthranil. Irradiation of TNBAL initially yields 2-nitroso-4,6-dinitrobenzoic acid and finally a dimer that decarboxylates to the monocarboxy white compound. The alcohol reacts in an analogous way, ultimately forming TNBAL and dimers, desoxy white compound, and the monocarboxy white compound. Kaplan et al. (1975) suggest that the dimers react further to form three- to four-ringed "insoluble" compounds.

Photolysis of TNT in an aqueous solution also produced an unidentified pink intermediate that slowly reverted to TNT in the dark (Mabey et al., 1983). This product did not promote photolysis of TNT as other products did. Also, Mabey et al. did not consider this intermediate to be the aci-TNT shown in Fig. 3-1 as structure I. It could, however, be a Meisenheimer compound (see Fig. 3-2).

The mechanism of TNT photolysis has not been fully established. Possible mechanisms consists of the 2,4,6-trinitrobenzyl anion pathway proposed by Burlinson et al. (1979b) or the triplet state proposed by Mabey et al. (1981). The photolysis products are both photosensitive themselves and act as catalysts for further TNT photolysis. Humic substances accelerate TNT photolysis, while oxygen retards TNT photolysis (Mabey et al., 1981). The photolysis results in the oxidation of the methyl group and finally decarboxylation.

Biotransformation

Many bacteria, yeasts, and fungi reduce the nitro groups in TNT to amines or azoxy dimers. The degree and rate of reduction vary with the types of organisms and environmental conditions. With appropriate conditions, all nitro groups can be converted, but reduction stops without mineralization of the aromatic ring (see Fig. 3-3.) Table 3-6 lists the biotransformation products that have been identified by various researchers.

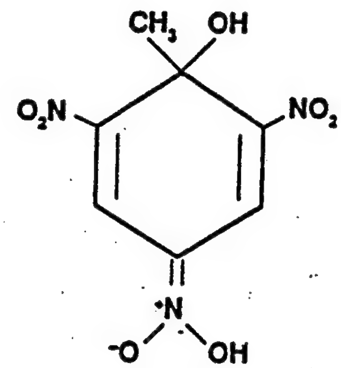


Fig. 3-2. Possible Meisenheimer compound formed by irradiation of TNT.

Microbes that have or can develop the ability to reduce TNT are common. Of the 190 fungi studied by Parrish (1977) for their ability to reduce TNT, only six could not. Spanggord et al. (1980b) studied organisms isolated from several types of water, and all reduced TNT. Osmon and Klausmeier (1973) found that many soils and water samples had microorganisms with TNT-reducing ability.

Biotransformation experiments using natural waters have been completed by Burlinson (1980) and Spanggord et al. (1980b). In the study by Burlinson (1980) 20 mg/L of TNT in raw Potomac River water was reduced by half over a period of 7.5 d. An adaptation period or lag period was not observed. Experiments conducted by Spanggord et al. (1980b) using natural waters (see Table 3-7) required small amounts of organic nutrient for cell growth (not for TNT biotransformation). They found that TNT biotransformation had a lag time of 13 to 40 d and a half-life of 8 to 25 d, depending on the water source.

Many researchers have studied microbial degradation of TNT in the presence of added nutrients and/or concentrated microbial populations. Nutrients increase the rates of the reactions and in some cases, enable recalcitrant reactions (i.e., mineralization) to proceed. Won et al. (1974) studied the influence of glucose and yeast on the biotransformation of TNT by a specially adapted pseudomonad organism. Although the rate of TNT loss in cultures without added nutrient was reported only on thin layer chromatograms, it was much obviously slower than the rate with 0.5% added glucose or yeast extract, based on a comparison of the chromatograms of the products at 24-h

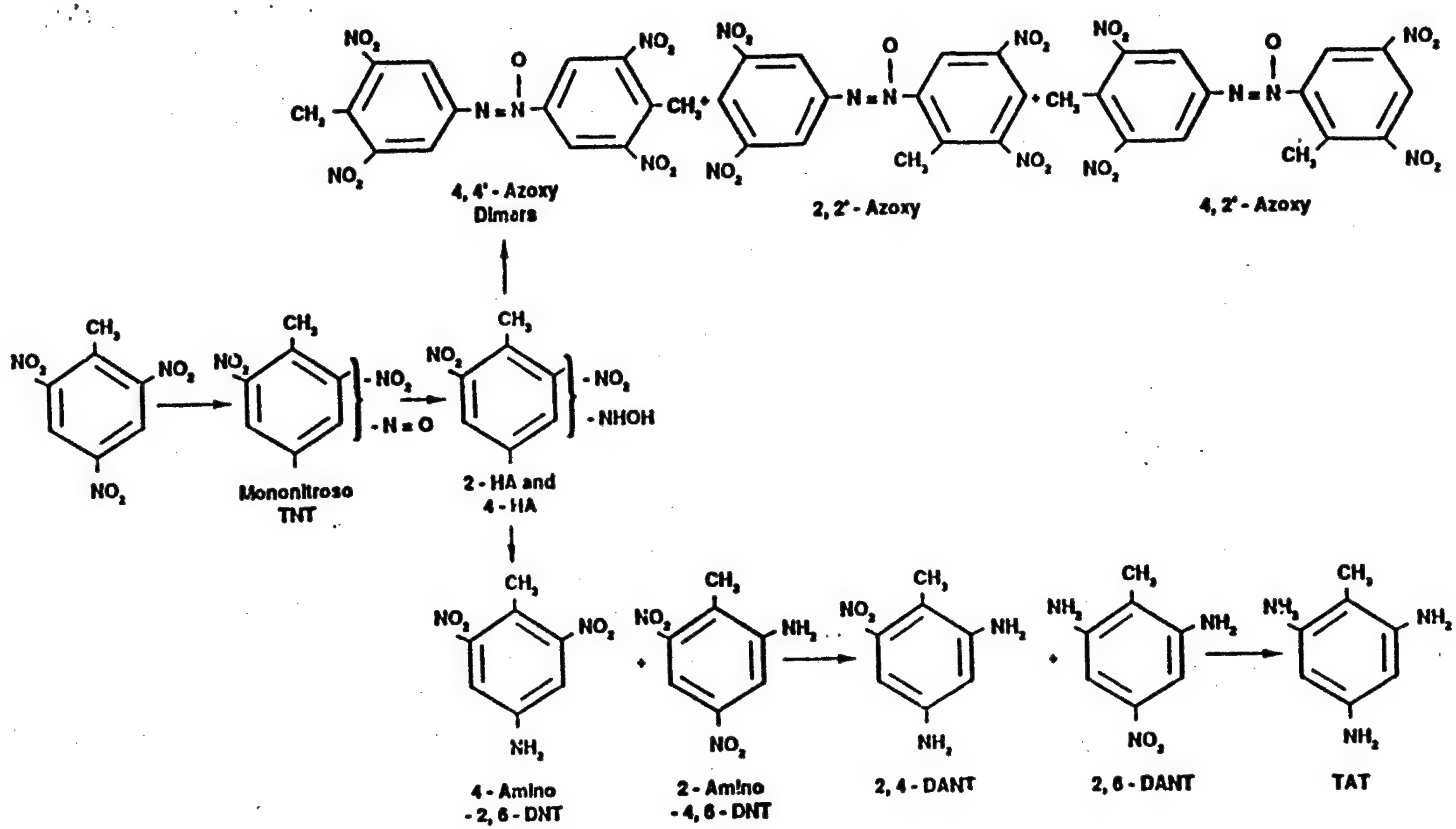


Figure 3-3. Biotransformation pathways of TNT.

Table 3-6. Biotransformation products of TNT that have been identified by various researchers.

Compound	References
4-amino-2,6-DNT	a,b,c,d,e,f,g
4-hydroxyamino-2,6-DNT	d,g
2-amino-4,6-DNT	a,b,c,e,f,g
2,4-DANT	b,c,d,f,g
2,6-DANT	b,c
TAT	d
4,4'-azoxy	c,d,f,g
2,2'-azoxy	d,g
2,4'-azoxy	c

[a] Burlinson, 1980.
[b] Hoffsommer _et_ _al_., 1978.
[c] Kaplan and Kaplan, 1982a.
[d] McCormick _et_ _al_., 1976.
[e] Naumova _et_ _al_., 1983.
[f] Spanggord _et_ _al_., 1980b.
[g] Won _et_ _al_., 1974.

intervals. Whereas a substantial amount of the initial TNT persisted in the solution without added nutrient after 96 h, TNT decreased to 1% of the original level within 24 h when 0.5% yeast was added to cultures. Also, these additional products were isolated when nutrients were present: 4-amino-DNT, 4-hydroxyamino-2,6-DNT and diamino-NT.

Yeast extract added to a basal mineral-salts medium enabled a variety of microorganisms to transform TNT in an aerobic environment. Osmon and Klausmeier (1973) tested microorganisms from sewage effluent; an effluent from a TNT loading facility; aquaria water; and soil for their ability to

Table 3-7. Biotransformation of 10 mg/L TNT in different sources of water (Spanggord et al., 1980b).

Water sample	Dissolved in DMSO[a]	Lag time (d)	Half-life (d)
Waconda Bay[b] water 1	*	20	25
Waconda Bay[b] water 2	*	13	19
Waconda Bay[b] water 3 with 1 wt% sediment	*	--	18
Searsville Pond[c] water	*	33	16
Searsville Pond[c] water		40	8

a TNT in solutions marked with an asterisk were dissolved in dimethylsulfoxide (DMSO) before addition to the water.

b Waconda Bay organisms have been previously exposed to TNT.

c Searsville Pond organisms have not been previously exposed to TNT.

biodegrade 100 mg/L TNT in a mineral-salts medium. In all cases, biotransformation was complete within 6 d. When the TNT-reducing organisms were isolated, the majority were "pseudomonas-like". Likewise, Kayser et al. (1977) found that organisms in local sewage sludge and "a pseudomonas strain isolated from the TNT contaminated streams at NAD, McAlester" were equivalent in their ability to reduce TNT in aqueous solution when supplemented with corn-steep nutrient.

Biotransformation products and rates are also affected by the presence of oxygen in the medium. Kayser et al. (1977) measured the loss of TNT in aerobic and anaerobic media supplemented with "corn-steep liquor". After 24 h, the aerobic sample lost 99.5% of the TNT. In the anaerobic medium, only 69% of the TNT disappeared. The anaerobic products contained three times as many amines as the aerobic products. McCormick et al. (1976) used five bacterial strains to study the effects of aerobic vs. anaerobic conditions, as well as cell growth phase, on biotransformation. A cell-free extract of three

anaerobic bacteria (*C. pasteurianum*, *V. alkalescens*, and *E. coli* (anaerobic)) reduced all three nitro groups of TNT to amines in a hydrogen atmosphere. When resting cells were used under the same conditions, *C. pasteurianum* and *V. alkalescens* again reduced TNT to its triamino analogue, whereas *E. coli* (anaerobic) reduced TNT only to the diamino compound. On the other hand, the two aerobic bacteria (*E. coli* (aerobic) and *Pseudomonad FR2*) reduced TNT no further than the diaminonitrotoluenes. They also observed that the dimer, 4,4'-azoxy was produced more frequently by the aerobic bacteria, even when grown in a hydrogen atmosphere.

The microbial degradation of TNT is directly affected by the pH of the medium. Kayser *et al*. (1977) found 99% of the TNT in batch-type experiments (using sewage sludge supplemented with nutrients) disappeared at pH 7.4 to 7.8, but only 45% at pH 6.0 to 6.2. Naumova *et al*. (1983) found a larger percentage of 2-amino-4,6-DNT in cell cultures that had been treated with TNT at a pH of 5.5 than at a pH of 7.8. With *Ps. denitrificans*, however, the effect of a pH increase from 5.5 to 7.8 was slight; the ratio of 2-amino-4,6-DNT to 4-amino-2,6-DNT changed from 3.9 to 3.2; whereas with *E. coli*, the ratio changed from 4.4 to 1.9.

Other researchers have reported different ratios of the bioreduction products. Parrish (1977) studied 190 fungal organisms for their ability to transform TNT and observed reduction solely in the 4-position of the benzene ring. Products included 4-amino-2,6-DNT, 4-hydroxyamino-2,6-DNT, and 4,4'-azoxy. Kayser *et al*. (1977) found unequal amounts of the amino-DNT isomers in their oxidation ditch facility using sludge microorganisms and corn-steep liquor (the 4-amino-2,6-DNT to 2-amino-4,6-DNT ratio was 8.3). On the other hand, Won *et al*. (1974) did not detect 4-amino-2,5-DNT in cultures of "pseudomonas-like organisms" used to degrade TNT. They did detect the 4,4'-azoxy dimer, presumably formed from the precursor to 4-amino-2,6-DNT. Naumova *et al*. (1983) did not measure the 4,4'-azoxy dimer in their cell cultures, but found that the ratio of the 2-amino-4,6-DNT to 4-amino-2,6-DNT varied from 5 to 1 in the exponential growth phase to nearly 2 to 1 in the stationary and drying-off phases. Because the 2- and 6- positions of TNT are equivalent, a random reduction would result in production of twice as much 2-amino-4,6-DNT as 4-amino-2,6-DNT (see Fig. 3-3). Their measurements were

made after 2 h growth with large bacterial populations of Pseudomonas denitrificans and Escherichia coli. In an earlier study, Naumova et al. (1982) found that the 2-amino isomer predominates in the bioconversion of TNT by fungi and yeasts as well.

Kaplan and Kaplan (1982a) investigated the bioreduction of TNT in compost at 55°C by thermophilic bacteria, Bacillus stearothermophilus Donle, B. subtilis Cohn, and B. coagulans Hammer, and a fungus, hyphomycete, Thermomyces languinosa Tsiklinslaya. Thermophilic biotransformation of TNT produced 2-amino-4,6-DNT, 4-amino-2,6-DNT, and 2,4'-azoxy compound. Five mesophilic bacteria used in a study by McCormick et al. (1976) produced the 4,4'-azoxy and 2,2'-azoxy isomers as well as 4-amino-2,6-DNT and 2,4-DANT.

The basic biotransformation pathway for TNT includes the reduction of a nitro group to a hydroxylamine, followed by either dimerization or further reduction. McCormick et al. (1976) measured the uptake of three molecules of hydrogen for the reduction of a nitro group to an amine as shown in Fig. 3-4. The complete sequence is shown in Fig. 3-3.

Under environmental conditions, mineralization of TNT is not expected to occur. Microbes degrade products in which the aromatic ring has intermediates containing hydroxyl groups ortho or para to each other (Dagley, 1975). In TNT, a dihydroxy intermediate could be produced by reduction of two nitro groups to amines, with subsequent substitution of the amines by hydroxyl ions. However, the dihydroxybenzene produced would have the hydroxy groups meta to each other instead of ortho or para, and hence would be unreactive.

Only very slight mineralization of TNT (0.4%) occurred when Kayser et al. (1977) measured ^{14}C-CO_2 produced in a composting experiment with corn-steep nutrients and sludge microorganisms. Likewise, Carpenter et al. (1978) found less than 0.5% ^{14}C-CO_2 in an activated sludge system after 3 to 5 d in an aerated reactor. Isbister et al. (1984) reported no CO_2 from TNT degradation in compost. Even when 1000 ppm yeast extract was grown with 10 ppm of radiolabeled TNT, less than 0.5% of the initial ^{14}C was present in CO_2 (Spanggord et al. (1980b). Only one study, Traxler (1976), reported that large inocula of bacterial cells from two unspecified sources metabolized ^{14}C-TNT to CO_2.

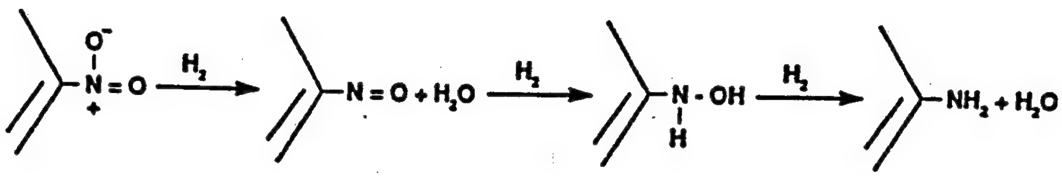


Figure 3-4. Reduction of a nitro group to an amine (adapted from Spanggord, 1980a).

In summary, many yeasts, fungi, and bacteria reduce the nitro groups of TNT. Products include dimers of reduction intermediates and mono-, di- and triamines. It appears that the 4-nitro group is attacked most readily, but it dimerizes in preference to reduction to the amine. Aerobic conditions produce mono- and diamines, whereas an anaerobic medium may produce triamines as well. At low pH's some bacteria produce more 2-amino-4,6-DNT than the 4-amino isomer. No cleavage of the aromatic ring is expected from microbial action. The maximum influence on reaction rates was observed by adding nutrients so that co-metabolism could result. The presence of oxygen and alkalinity increase the microbial reduction rate only slightly. Environmental half-lives for microbial reduction of TNT in surface water and possibly moist soils are expected to be from one week to a month with a possible lag time of up to a month (see Table 3-7).

Compound and Complex Formation

Although the microbial transformation products of TNT are not mineralized in natural waters, they do appear to degrade. McCormick (private communication, 1986) suggests that microbial reduction of TNT produces highly substituted anilines or their precursors, which subsequently react with carboxyl groups of humic acids, lipids, or proteins to form insoluble precipitates. In research that supports this process, Kaplan and Kaplan (1983) performed tests of the binding of humic acids with TNT and two amino

reduction products, namely, 2,4-dinitroaniline (2,4-DNA) and 2,6-dinitroaniline (2,6-DNA). They concluded that TNT itself did not bind to the humic acids, but the two amines bound readily. The binding reactions were enhanced at increased acidity (i.e., pH 4), where the amines were also less stable. The fate or toxicity of the complexes is unknown.

Carpenter et al. (1978) provided additional evidence for complex formation in their study of the microbial degradation of ^{14}C-TNT in an activated-sludge system. They found that the ^{14}C activity in the lipid and protein fractions of the product was associated with insoluble precipitates. Infrared spectra indicated that the precipitates were polyamide-type macromolecules that were resistant to further biodegradation. Virtually all of the radioactivity was contained in the sludge pellet after centrifuging. Several other composting experiments have shown that the TNT microbial products are nonextractable from the organic compost material. For example, Kaplan and Kaplan (1982a) studied TNT degradation in compost and found progressively more TNT degradation products were bound to the organic matter after the compost had been cured and stabilized. Likewise, Isbister et al. (1984) found that ^{14}C-TNT was rapidly transformed into humus-like materials (1/2 of the ^{14}C-TNT in compost was not extractable after 3 wk). They found increasing concentrations of [^{14}C] in the compost solids. After 6 weeks, they could extract only an average of 19.7% of the label with benzene/methanol and benzene; in contrast, 66.5% and the [^{14}C] was in the compost solids (only 86.9% of the label was recovered). Hoffsommer et al. (1978) performed experiments in an "oxidation ditch facility" to "find an efficient biological process for destroying TNT in waste water." They suggested that TNT is absorbed into the bacterial floc, where it is reduced to its amino analogues. The bacteria subsequently die and the amines are slowly extracted, or the remaining nitro groups are reduced further by other microorganisms. Their paper describes other experiments that implied that the amines continued to degrade to polar compounds. Using a closed system, they added ^{14}C-TNT to nutrient- and microorganism-rich water. After three days, aminodinitrotoluenes accounted for 26% of the ^{14}C activity in the aqueous phase and 8% of the activity in or on the bacterial floc. After 30 d, amines only accounted for 0.28% of the activity in the supernatant and 2.8% in the floc. When both the floc and the supernatant were extracted with benzene after 3 and 30 d, the total nonextractable ^{14}C increased from 50.5 to 90.8% (clearly some complexes or compounds were formed). The latter figure included 33.2% in the

supernatant (probably polar substances) and 57.6% in or on the floc. Similarly, Spanggord et al. (1980b) recovered only 30 to 40% of ^{14}C-TNT after 240 h from unsterilized, unpreserved sediment, while measuring sediment/water partition coefficients. They suggested that their low recovery might result from biodegradation, irreversible sorption, or reactions with the sediment. When the sediment was autoclaved and $HgCl_2$ was added, ^{14}C-TNT recovery was increased to 90%.

Hydrolysis

Hydrolysis is not expected to be an environmental degradation pathway of TNT. Although hydrolysis of TNT does occur at high pH (Urbanski, 1986a), it does not occur in seawater that has a pH of approximately 8 (Hoffsommer and Rosen, 1973).

TOXIC EFFECTS ON ANIMALS AND HUMANS

Several literature reviews have dealt with the toxic effects of TNT. Included among these reviews are Hathaway (1977), Zakhari et al. (1978), and Ryon et al. (1984). The primary intent of this assessment is to discuss the salient toxicological properties of TNT and to assess dose-response data available for determining dose rates that would safeguard the health of individuals exposed to contaminated foods, soils, etc. In the first part of our assessment we examine pertinent studies dealing with toxicokinetics, followed by a review of toxic effects to major organs and systems. Subsequent sections discuss the teratogenicity, genotoxicity, and carcinogenicity of TNT. The final subsection includes an analysis of dose-response data for quantifying safe intakes.

Toxicokinetics

TNT metabolism has been studied by a number of investigators including Putnam and Herman (1919), Voegtlin et al. (1921a, 1921b), Lee et al. (1975), Hodgson et al. (1977), and El-hawari et al. (1978). Information is available on its absorption, rate of excretion and pathways of metabolism. In addition, many of the TNT metabolites have been identified.

12. DATA-BASE ASSESSMENT FOR TETRYL

Our review of the available literature on tetryl, a booster explosive, indicates that there are major gaps in our knowledge of its environmental chemistry and toxicology. Although some aspects of its potential behavior in the environment have been studied recently (i.e., hydrolysis and photolysis), no work has addressed biotransformation processes. Moreover, there is little information on dose-response relationships for different toxic endpoints in humans and laboratory animals -- and thus our ability to derive an acceptable daily intake to protect human health is limited.

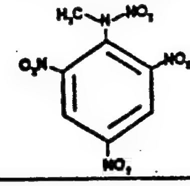
ENVIRONMENTAL PARTITIONING AND TRANSPORT PROPERTIES

Table 12-1 presents the structural formula and other identifiers for tetryl; chemical and physical properties are provided in Table 12-2; and partition coefficients are presented in Table 12-3.

Information on the transport of tetryl in soils is limited primarily to the work of Hale et al. (1979), who studied the migration of tetryl through soil columns. Kayser and Burlinson (1982), in follow-on work, analyzed soil and water samples from the experiments of Hale et al. to obtain additional information on the environmental chemistry of tetryl. After 20 weeks, ^{14}C-tetryl was not detected in the effluent of 5 cm-diameter, 61-cm-long soil columns irrigated at 5 cm per week (Kayser and Burlinson, 1982; Hale et al., 1979). However, examination of the effluent collected periodically showed ^{14}C-labeled compounds as decomposition products. The product found in the largest amount was picric acid (a hydrolysis by-product of tetryl) and the remainder was judged to be composed of polar compounds that were water soluble and nonvolatile (Kayser and Burlinson, 1982).

Hale et al. (1979) also measured the partitioning of tetryl between water and each of the four soils used in the column experiments. The K_d values for the soils (measured by a shake-flask technique) ranged from 7.6 to 35.3 (see Table 12-4). To estimate the K_{oc} values for the soils, we divided the K_d values by the average organic-carbon (OC) content, calculated from data for different column intervals. The average OC values ranged from 0.56 wt% for the Bennington soil to 2.2 wt% for the Genesee soil. The K_{oc} values ranged from 1357 to 2948 (see Table 12-4). Our calculated K_{oc} value, in contrast,

Table 12-1. Chemical identifiers of tetryl.

Empirical formula:	$C_7H_5N_5O_8$	Structural formula: 
Molecular weight:	287.15	
CAS reg. no.:	[479-45-8]	
RTECS no.:	BY6300000	

was 271 (Table 12-3). One explanation for the higher measured K_{oc} values is that the methylnitroamino group of tetryl may be binding or complexing with humic materials. Evidence supporting this binding phenomenom comes from Bongiovanni et al. (1984) who found that tetryl was more difficult extract at low concentrations in soil (i.e., 5 ppm) than other munitions. With one exception, Hale et al. (1979) measured K_ds that were an order of magnitude greater if the soil concentration was derived from the difference between the water concentration before and after partitioning, instead of the soil concentration measured by direct extraction of the soil. In other work, Lakings and Gan (1981) could not extract tetryl from plant tissue matrices, whereas they could extract other munitions with the same methods. They concluded that tetryl was irreversibly adsorbed to protein or other macromolecules. We suspect that the methylnitroamino group reacts with macromolecules in the same way that the amine microbial degradation products of TNT do (see Section 3).

The equilibrium distribution of tetryl, as shown in Table 12-5, ought to be directly affected by its low Henry's law constant (among the lowest of the explosives at 1.5×10^{-6} L-torr/mol) and a moderately high K_{oc} value (i.e., greater than 271). More tetryl (three to ten percent) than the other explosives and co-contaminants examined should reside in the upper soil zone.

ENVIRONMENTAL TRANSFORMATION PROCESSES

The environmental fate of tetryl parallels that of TNT because of the structural similarity of the two compounds. For example, both have comparable photolysis rates (a few days). Tetryl, however, has a functional group, the methylnitroamino group, that is subject to hydrolysis. Also, because of this methylnitroamino group, we suspect that tetryl binds to naturally occurring

Table 12-2. Chemical and physical properties of tetryl. Properties are for an ambient temperature of 20°C.

Property	Units	Value	Source
Boiling point	°C	Explodes at 180 to 190	Windholz *et al.*, 1983
Freezing/melting point	°C	130 to 132	Windholz *et al.*, 1983
Density	g/mL	1.73	Lindner, 1980
Vapor pressure	torr	4×10^{-10}	Calculated[a] from a vapor pressure-temperature equation
Aqueous solubility	mol/L mg/L	2.6×10^{-4} 75	Taylor and Rinkenback, 1923
Henry's law constant	(L-torr)/mol	2×10^{-6}	Calculated as the ratio of vapor pressure to aqueous solubility.
Diffusion coefficients: Air Water	m^2/s m^2/s	5.3×10^{-6} 5.1×10^{-10}	Estimated[a] Estimated[a]
Log octanol/water partition coefficient (K_{OW})	log K_{OW}	2	Estimated[a]

[a] See Appendix A.

Table 12-3. Partition coefficients for use in estimating human exposures to tetryl.

Partition coefficient	Parameter	Units	Value	Source
Plant/soil	K_{sp}	(mg/kg plant)[a]/(mg/kg soil)	1	Calculated [a]from molecular weight
Beef-fat/diet	K_{fd}	(ppm in fat)/(ppm diet)	3.7×10^{-3}	Average of values calculated[b] from water solubility and log K_{ow}
Fish/water	K_{fw}	(mg/kg fish)[a]/(mg/kg water)	15	Calculated[b] from log K_{ow}
SOC/water	K_{oc}	(mg/kg SOC/(mg/kg water)	270	Estimated[b] from solubility in water and melting point.

[a] Het-weights for plant and fish

[b] See Appendix A

Table 12-4. Partitioning of tetryl between water and soils (data from Hale et al., 1979)

Soil type	K_d	Organic carbon, wt%	K_{oc}
Brookston	34.3	1.8	1906
Genesee	35.3	2.2	1604
Princeton	11.5	0.39	2948
Bennington	7.6	0.56	1357

macromolecules, lipids, and proteins; whereas only the microbial degradation products of TNT (i.e., 2-A-4,6-DNT and 4-A-2,6-DNT), but not TNT itself, bind or form complexes with these compounds. No information was found on the microbial degradation of tetryl.

Photolysis

Photolysis appears to be a major environmental transformation process for tetryl. Kayser et al. (1984), in studying the hydrolysis of tetryl, found that it disappeared with laboratory room light at a rate that was at least an order of magnitude greater than the hydrolysis loss rate in the dark. The experimental conditions were distilled water and "ambient" temperature. They found that 95% of the tetryl disappeared after 20 d; from these figures we calculate a half-life of 4.5 d. Sunlight photolysis is expected to be faster because of the increased intensity of the light.

Hydrolysis

Hydrolysis is a major environmental fate of tetryl in the absence of light. Kayser et al. (1984) measured the hydrolysis of tetryl in distilled water at a pH of 6.8 and a temperature of 40°C. The calculated half-life was 63 d. They estimated the half-life at 20°C to be about 300 d. Several of their preliminary measurements gave similar half-lives (see Table 12-6).

Table 12-5. Fractional distributions of tetryl among the eight compartments of environmental landscapes representing areas in the U.S. where demilitarization operations occur. Distributions are based on the steady-state addition of tetryl to the upper-soil layer. For details of the compartments, see Appendix B.

Compartment	Western ecoregion	Southeastern ecoregion
	Fraction[a] of total inventory present	
Air	1.1×10^{-12}	1.3×10^{-12}
Air particles	7.1×10^{-9}	1.6×10^{-8}
Biota	6.9×10^{-5}	0.0068
Upper soil	0.030	0.10
Lower soil	0.43	0.099
Ground water	0.54	0.78
Surface water	0.0019	0.0068
Sediments	1.3×10^{-4}	4.7×10^{-4}

[a] Fractions do not add to one because of rounding.

The hydrolysis reaction is second order in hydroxide ion concentration (Kayser et al., 1984), and therefore, a pH change from 7 to 8 should increase the rate by an order of magnitude. The hydrolytic degradation rates determined by Kayser et al. (1984) agree with the measurements of Hoffsommer and Rosen (1973) for tetryl in seawater (pH = 8). Hoffsommer and Rosen found that 88% of the tetryl had hydrolyzed after 101 d. The associated half-life for hydrolysis at pH 8 is approximately 33 d, which is an order of magnitude shorter than that at pH 7 (see Table 12-6).

Biotransformation

No information was found on the microbial degradation of tetryl. However, it is reasonable to assume that microbial action will reduce the nitro groups to amines.

Table 12-6. Hydrolysis half-lives of tetryl.

Concentration (mg/L)	pH	Experimental conditions	Half-life (d)
12	6	Distilled water in the dark at an "ambient temperature" 3.2% of the tetryl was lost in 20 d[a]	430
15	6	Distilled water in the dark at an "ambient temperature." 5.1% of the tetryl was lost in 20 d[a]	260
1 to 20	6.8	Distilled water at 40°C[a]	63
26	8	Seawater at 25°C., 88% of the tetryl disappeared in 101 d[b]	33

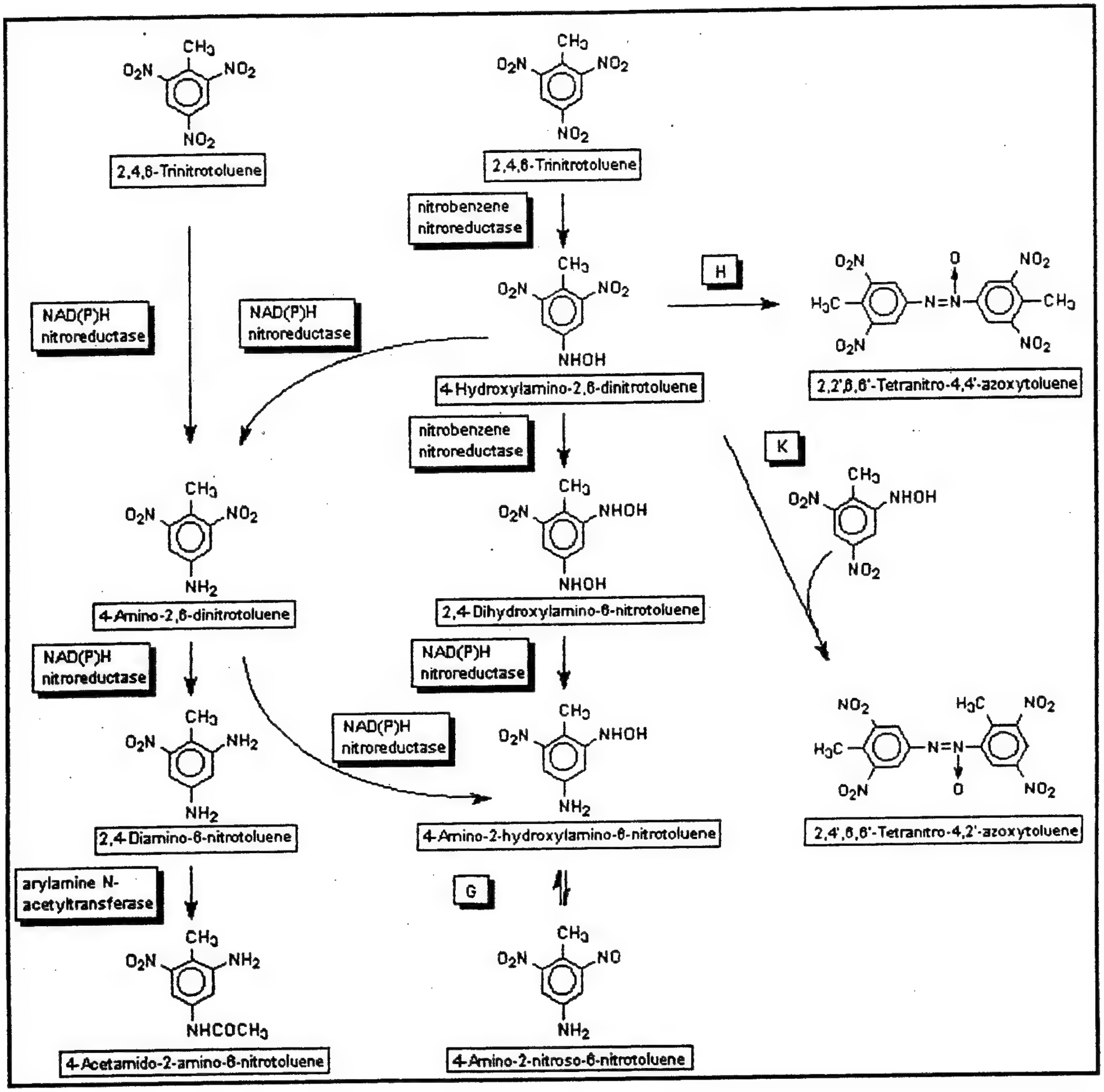
[a] Kayser et al., 1984.
[b] Hoffsommer and Rosen, 1973.

TOXIC EFFECTS ON ANIMALS AND HUMANS

Much of the toxicity data available on tetryl is based on occupational exposures associated with its production in ordnance factories during the 1930's and 1940's. Exposures to tetryl dust particles occurred most frequently among workers who were either blending tetryl with graphite in the loading plants, inserting the tetryl into booster bags, or packing it for shipment (Schwartz, 1944). The information derived from the occupational exposures is qualitative in nature. Few animal studies have been completed in the last 20 years, and most of the recent work on this explosive has focused on its genotoxicity.

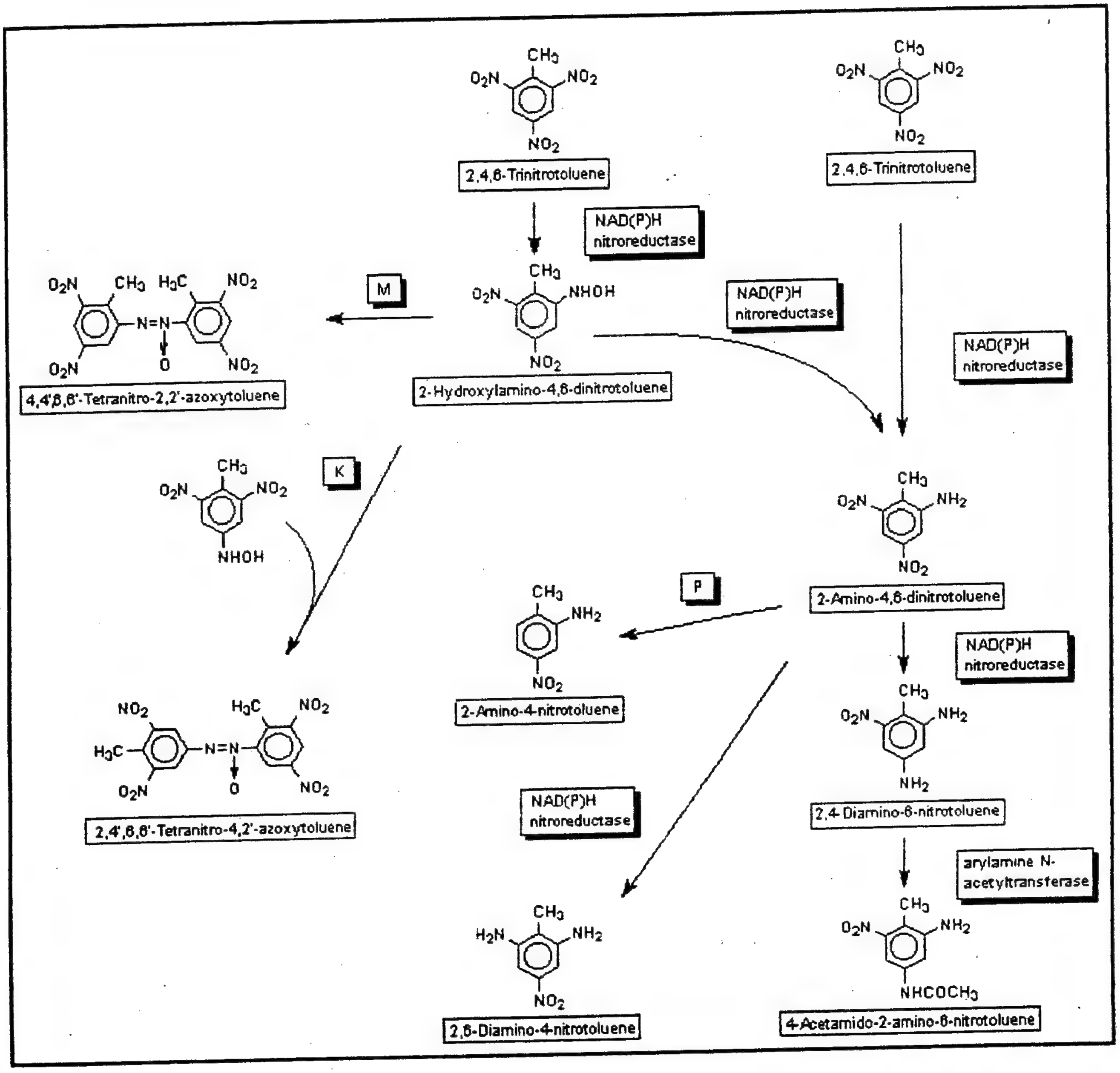
Toxicokinetics

There are no data available that deal directly with the toxicokinetics of tetryl.



2,4,6-Trinitrotoluene Aerobic Pathway (1)
Anti-Aircraft Artillery Ranges Sampling and Analysis Plan
Fort Sheridan, Illinois

ESE
A MACTEC COMPANY



2,4,6-Trinitrotoluene Aerobic Pathway (2)
Anti-Aircraft Artillery Ranges Sampling and Analysis Plan
Fort Sheridan, Illinois

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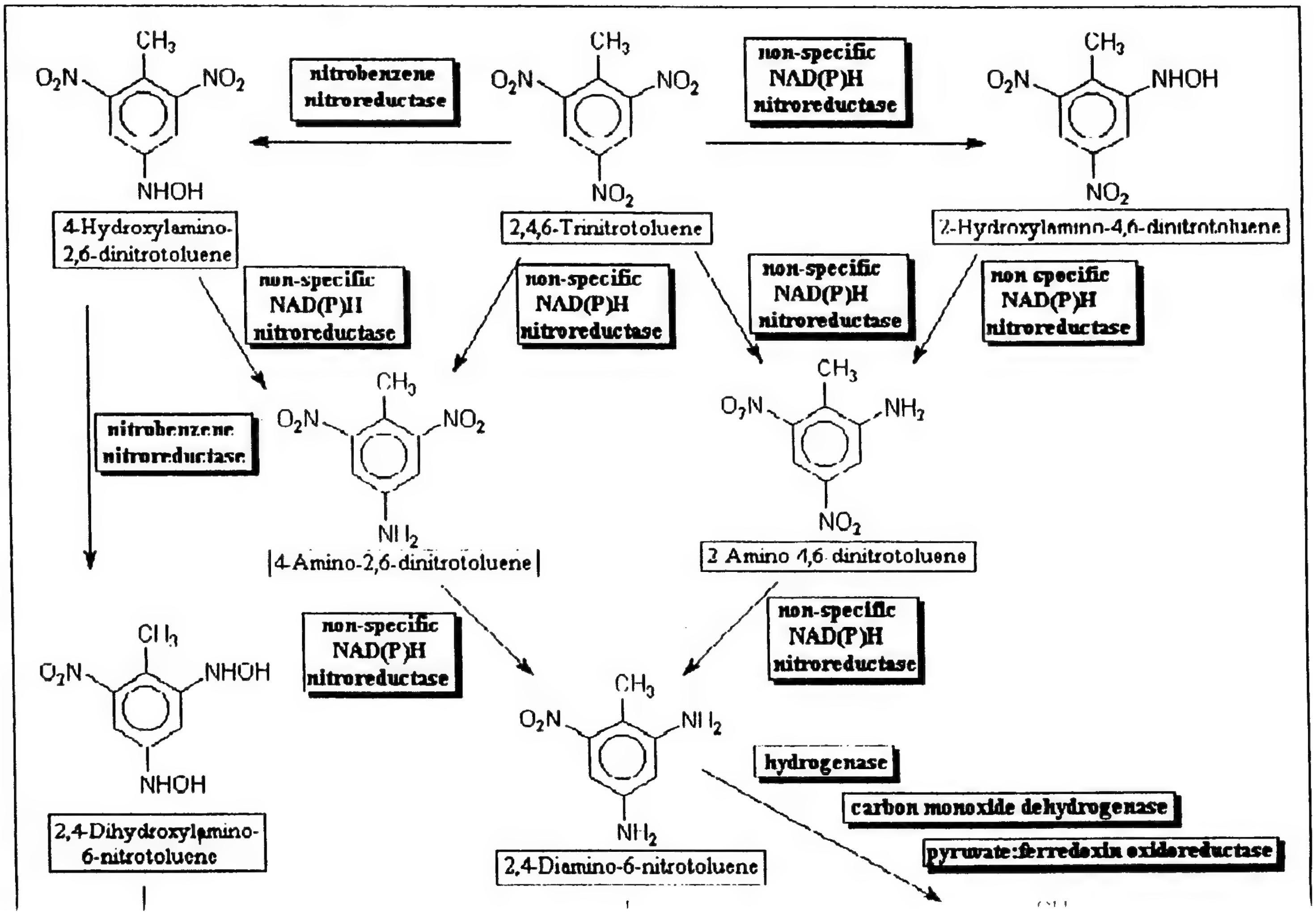
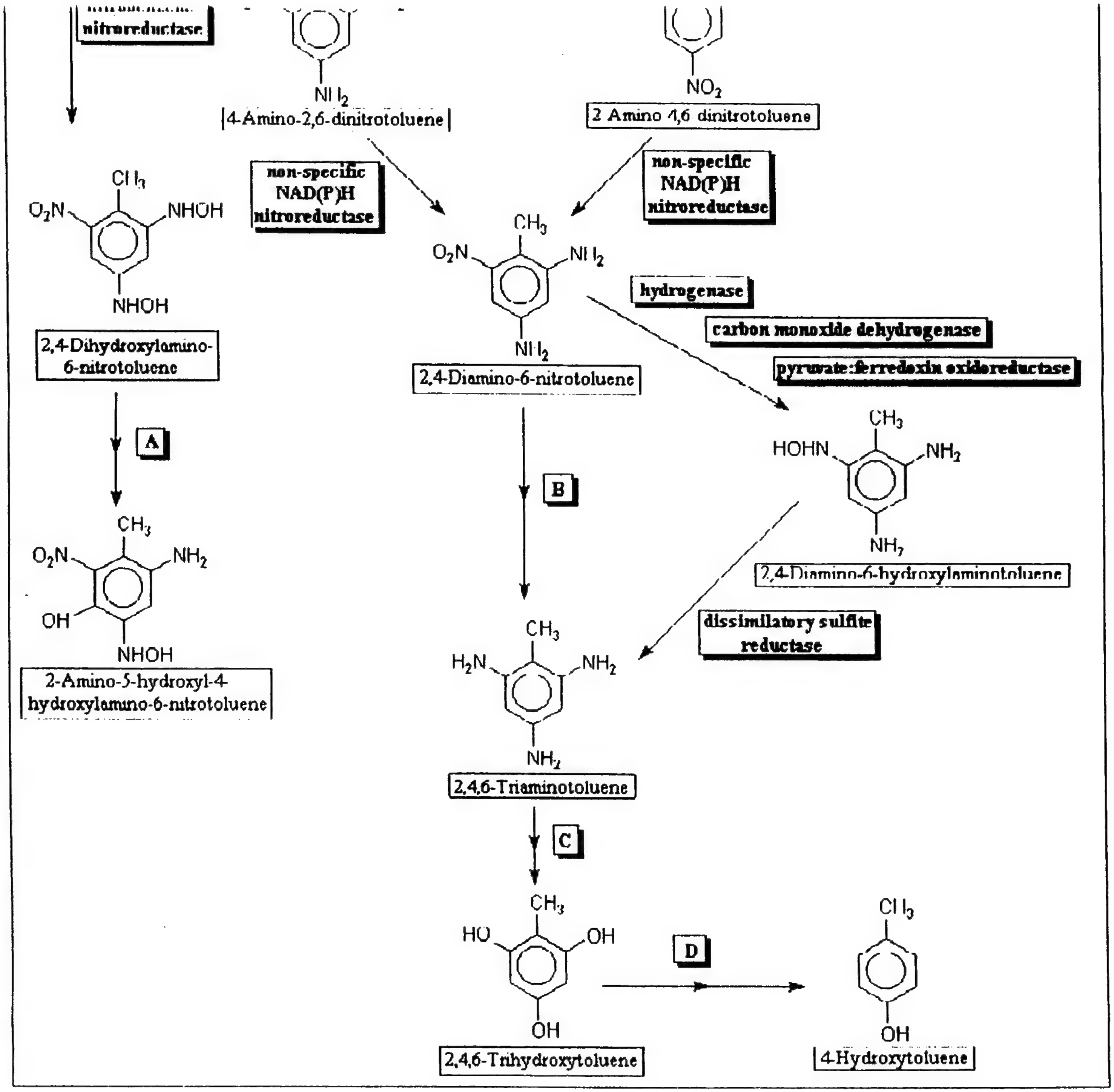
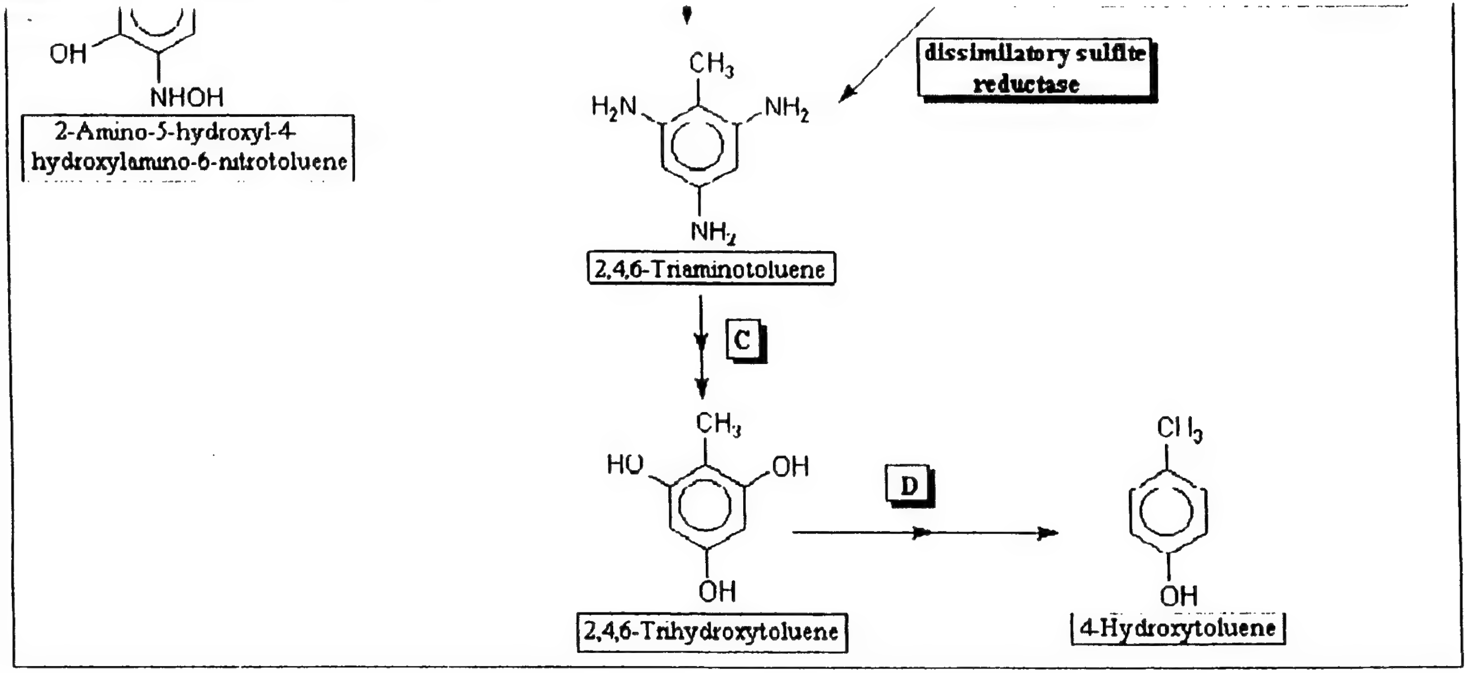

nitrobenzene nitroreductase
non-specific NAD(P)H nitroreductase
4-Hydroxylamino-2,6-dinitrotoluene
2,4,6-Trinitrotoluene
2-Hydroxylamino-4,6-dinitrotoluene
4-Amino-2,6-dinitrotoluene
2 Amino 4,6 dinitrotoluene
2,4-Dihydroxylamino-6-nitrotoluene
2,4-Diamino-6-nitrotoluene
hydrogenase
carbon monoxide dehydrogenase
pyruvate:ferredoxin oxidoreductase



nitroreductase
4-Amino-2,6-dinitrotoluene
2 Amino 4,6 dinitrotoluene
non-specific NAD(P)H nitroreductase
non-specific NAD(P)H nitroreductase
2,4-Dihydroxylamino-6-nitrotoluene
2,4-Diamino-6-nitrotoluene
hydrogenase
carbon monoxide dehydrogenase
pyruvate:ferredoxin oxidoreductase
A
B
2,4-Diamino-6-hydroxylaminotoluene
2-Amino-5-hydroxyl-4-hydroxylamino-6-nitrotoluene
dissimilatory sulfite reductase
2,4,6-Triaminotoluene
C
D
2,4,6-Trihydroxytoluene
4-Hydroxytoluene



3

2,4,6-Trinitrotoluene Anaerobic Pathway
Anti-Aircraft Artillery Ranges Sampling and Analysis Plan
Fort Sheridan, Illinois

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Appendix C

Equipment List

Equipment List

Health and Safety Equipment

Sampling Equipment

- Stainless-Steel Spoons/Trowels
- Stainless-Steel Bowls
- Petite Ponar Dredge
- Eckman Dredge

Miscellaneous

- Ordnance Locator (MK26 Ordnance Locator) or equivalent
- Trimble Navigation GPS

Appendix D

SW-846 Methods 8000B and 8330

METHOD 8000B

DETERMINATIVE CHROMATOGRAPHIC SEPARATIONS

1.0 SCOPE AND APPLICATION

1.1 Method 8000 is not a determinative method but instead provides guidance on analytical chromatography and describes calibration and quality control requirements that are common to all SW-846 chromatographic methods. Apply Method 8000 in conjunction with all SW-846 determinative chromatographic methods. The methods include, but are not limited to, the following:

Method Number	Analytes	Chromatographic Technique (see Sec. 1.5)	Detector
7580	White phosphorus (P_4)	GC, capillary column	NPD
8011	EDB, DBCP	GC, capillary column	ECD
8015	Nonhalogenated volatiles	GC, packed & capillary column	FID
8021	Volatiles	GC, capillary column	PID, ELCD
8031	Acrylonitrile	GC, packed column	NPD
8032	Acrylamide	GC, packed column	ECD
8033	Acetonitrile	GC, capillary column	NPD
8041	Phenols	Underivatized or derivatized, GC, capillary column	FID, ECD
8061	Phthalates	GC, capillary column	ECD
8070	Nitrosamines	GC, packed column	NPD, ELCD, TED
8081	Organochlorine pesticides	GC, capillary column	ECD, ELCD
8082	Polychlorinated biphenyls	GC, capillary column	ECD, ELCD
8091	Nitroaromatics and cyclic ketones	GC, capillary column	ECD
8100	PAHs	GC, packed & capillary column	FID
8111	Haloethers	GC, capillary column	ECD
8121	Chlorinated hydrocarbons	GC, capillary column	ECD

Method Number	Analytes	Chromatographic Technique (see Sec. 1.5)	Detector
8131	Aniline and selected derivatives	GC, capillary column	NPD
8141	Organophosphorus pesticides	GC, capillary column	FPD, NPD, ELCD
8151	Acid herbicides	Derivatize; GC, capillary column	ECD
8260	Volatiles	GC, capillary column	MS
8270	Semivolatiles	GC, capillary column	MS
8275	Semivolatiles	Thermal extraction/GC	MS
8280	Dioxins and Dibenzofurans	GC, capillary column	Low resolution MS
8290	Dioxins and Dibenzofurans	GC, capillary column	High resolution MS
8310	PAHs	HPLC, reverse phase	UV, Fluorescence
8315	Carbonyl compounds	Derivatize; HPLC	Fluorescence
8316	Acrylamide, acrylonitrile, acrolein	HPLC, reverse phase	UV
8318	N-Methyl carbamates	Derivatize; HPLC	Fluorescence
8321	Extractable nonvolatiles	HPLC, reverse phase	TS/MS, UV
8325	Extractable nonvolatiles	HPLC, reverse phase	PB/MS, UV
8330	Nitroaromatics and nitramines	HPLC, reverse phase	UV
8331	Tetrazene	HPLC, ion pair, reverse phase	UV
8332	Nitroglycerine	HPLC, reverse phase	UV
8410	Semivolatiles	GC, capillary column	FT-IR
8430	Bis(2-chloroethyl) ether hydrolysis products	GC, capillary column	FT-IR

DBCP = Dibromochloropropane
ECD = Electron capture detector
EDB = Ethylene dibromide
ELCD = Electrolytic conductivity detector
FID = Flame ionization detector
FPD = Flame photometric detector
FT-IR = Fourier transform-infrared
GC = Gas chromatography
HPLC = High performance liquid chromatography
MS = Mass spectrometry
NPD = Nitrogen/phosphorous detector
PAHs = Polynuclear aromatic hydrocarbons
PB/MS = Particle beam mass spectrometry
PID = Photoionization detector
TED = Thermionic emission detector
TS/MS = Thermospray mass spectrometry
UV = Ultraviolet

1.2 Analytical chromatography is used to separate target analytes from co-extracted interferences in samples. Chromatographic methods can be divided into two major categories: gas chromatography (GC) and high performance liquid chromatography (HPLC).

1.2.1 Gas chromatography (more properly called gas-liquid chromatography) is the separation technique of choice for organic compounds which can be volatilized without being decomposed or chemically rearranged.

1.2.2 High performance liquid chromatography (HPLC) is a separation technique useful for semivolatile and nonvolatile chemicals or for analytes that decompose upon heating. Successful liquid chromatographic separation requires that the analyte(s) of interest be soluble in the solvent(s) selected for use as the mobile phase. Because the solvents are delivered under pressure, the technique was originally designated as high pressure liquid chromatography, but now is commonly referred to as high performance liquid chromatography.

1.3 All chromatographic processes achieve separation by passing a mobile phase over a stationary phase. Constituents in a mixture are separated because they partition differently between the mobile and stationary phases and thus have different retention times. Compounds that interact strongly with the stationary phase elute slowly (i.e., long retention time), while compounds that remain in the mobile phase elute quickly (i.e., short retention time).

1.3.1 The mobile phase for GC is an inert gas, usually helium, and the stationary phase is generally a silicone oil or similar material.

1.3.2 In "normal phase" HPLC, the mobile phase is less polar than the stationary phase. In "reverse phase" HPLC, the converse is true. Reverse phase HPLC is the technique of choice for environmental and waste analyses of non-volatile organic target analytes.

1.4 A number of specific GC and LC techniques are used for environmental and waste analyses. The specific techniques are distinguished by the chromatographic hardware or by the chemical mechanisms used to achieve separations.

1.4.1 GC methods, including those in SW-846, can be categorized on the basis of the chromatographic columns employed.

1.4.1.1 Packed columns are typically made from glass or stainless steel tubing and generally are 1.5 - 3 m long with a 2 - 4 mm ID, and filled with small particles (60-100 mesh diatomaceous earth or carbon) coated with a liquid phase.

1.4.1.2 Capillary columns are typically made from open tubular glass capillary columns that are 15 - 100 m long with a 0.2 - 0.75 mm ID, and coated with a liquid phase. Most capillary columns are now made of fused silica, although glass columns are still sold for the analysis of volatiles. Capillary columns are inherently more efficient than packed columns and have replaced packed columns for most SW-846 applications.

1.4.2 SW-846 HPLC methods are categorized on the basis of the mechanism of separation.

1.4.2.1 Partition chromatography is the basis of reverse phase HPLC separations. Analytes are separated on a hydrophobic column using a polar mobile phase pumped at high pressure (800 - 4000 psi) through a stainless steel column 10 -

25 cm long with a 2 - 4 mm ID and packed with 3 - 10 μm silica or divinyl benzene-styrene particles.

1.4.2.2 Ion exchange chromatography is used to separate ionic species.

1.5 SW-846 methods describe columns and conditions that have been demonstrated to provide optimum separation of all or most target analytes listed in that specific procedure. Most often, those columns were the ones used by EPA during method development and testing. Analysts may change those columns and conditions, provided that they demonstrate performance for the analytes of interest that is appropriate for the intended application. This is especially true when limited groups of analytes are to be monitored (i.e., if only a subset of the list of target analytes in a method are required, then the chromatographic conditions and columns may be optimized for those analytes).

1.5.1 Chromatographic performance is demonstrated by the resolution of standards and the ability to model the response of the detector during calibration, and by the sensitivity, accuracy, precision, frequency of false positives, and frequency of false negatives during analysis. The laboratory must demonstrate that an alternate chromatographic procedure provides performance that satisfies the analytical requirements of the specific application for which it is being used. Such demonstrations should be performed using the procedures outlined in Secs. 8.2 to 8.5 of this method and those in Chapter One.

1.5.2 In addition, laboratories must be cautious whenever the use of two dissimilar columns is included in a method for confirmation of identification. For instance, a DB-5 column generally cannot be used for confirmation of results obtained using an SPB-5 column because the stationary phases are not sufficiently dissimilar and the changes in elution order (if any) will not provide adequate confirmation.

1.6 When gas chromatographic conditions are changed, retention times and analytical separations are often affected. For example, increasing the GC oven temperature changes the partitioning between the mobile and stationary phases, leading to shorter retention times. GC retention times can also be changed by selecting a column with a different length, stationary-phase loading (i.e., capillary film thickness or percent loading for packed columns), or alternate liquid phase. As a result, two critical aspects of any SW-846 chromatographic method are the determination and/or verification of retention times and analyte separation.

1.7 HPLC retention times and analytical separations are also affected by changes in the mobile and stationary phases. The HPLC mobile phase is easily changed by adjusting the composition of the solvent mixture being pumped through the column. In reverse phase HPLC, increasing the ratio of methanol (or acetonitrile) to water shortens retention times. HPLC retention times can also be changed by selecting a column with (1) a different length, (2) an alternate bonded phase, or (3) a different particle size (e.g., smaller particles generally increase column resolution). SW-846 methods provide conditions that have been demonstrated to provide good HPLC separations using specific instruments to analyze a limited number of samples. Analysts (particularly those using HPLC/MS) may need to tailor the chromatographic conditions listed in the method for their specific application and/or instrument. HPLC methods are particularly sensitive to small changes in chromatographic conditions, including temperature. HPLC column temperature control ovens should be used to maintain constant retention times since ambient laboratory temperatures often fluctuate throughout the course of a day.

1.8 Chromatographic methods can be used to produce data of appropriate quality for the analysis of environmental and waste samples. However, data quality can be greatly enhanced when

the analyst understands both the intended use of the results and the limitations of the specific analytical procedures being employed. Therefore, these methods are recommended for use only by, or under the close supervision of, experienced analysts. Many difficulties observed in the performance of SW-846 methods for the analysis of RCRA wastes can be attributed to the lack of skill and training of the analyst.

1.8.1 Methods using selective (e.g., PID, NPD, ELCD) or non-selective (e.g., FID) detectors may present serious difficulties when used for site investigations, including co-elution of target analytes, false negatives due to retention time shifts, and false positives and quantitation errors due to co-eluting non-target sample components.

1.8.2 In contrast, GC methods employing selective or non-selective detectors may be appropriate for remediation activities where the analytes of concern are known, of limited number, and of significantly greater concentration than potentially interfering materials.

1.8.3 If the site is not well characterized, and especially if large numbers of target analytes are of concern, analysis by GC/MS or HPLC/MS may be more appropriate.

1.9 Each of the chromatographic methods includes a list of the compounds that it may be used to determine. The lists in some methods are lengthy and it may not be practical or appropriate to attempt to determine all the analytes simultaneously. Such analyte lists do not imply a regulatory requirement for the analysis of any or all of the compounds, but rather, indicate the method(s) which may be applicable to those analytes.

1.10 Prior to employing this method, analysts are advised to consult the disclaimer statement at the front of the manual and the information in Chapter Two for guidance on the allowed flexibility in the choice of apparatus, reagents, and supplies. In addition, unless specified in a regulation, the use of SW-846 methods is not mandatory in response to Federal testing requirements. The information contained in this procedure is provided by EPA as guidance to be used by the analyst and the regulated community in making judgments necessary to meet the data quality objectives or needs for the intended use of the data.

2.0 SUMMARY OF METHOD

Method 8000 describes general considerations in achieving chromatographic separations and performing calibrations. Method 8000 is to be used in conjunction with all SW-846 determinative chromatographic methods, including, but not limited to, each method listed in Sec. 1.1. Each of these chromatographic methods recommends appropriate procedures for sample preparation, extraction, cleanup, and/or derivatization. Consult the specific procedures for additional information on these crucial steps in the analytical process.

2.1 Sec. 3.1 of this method provides general guidance on minimizing contamination, including cross-contamination between samples. Sample screening procedures are strongly recommended, and discussed in Sec. 3.2.

2.2 Before any sample or blank is introduced into a chromatographic system, the appropriate resolution criteria and calibration procedure(s) described in Method 8000 must be satisfied (see Secs. 3.3 and 8.3).

2.3 Secs. 3.4 and 3.5 provide information on the effects of chromatographic interferences.

2.4 Sec 4.0 of this method contains generalized specifications for the components of both GC and HPLC systems used in SW-846 analyses.

2.5 Calibration of the analytical system is another critical step in the generation of quality data. Sec. 7.5 discusses specific procedures and calculations for both linear and non-linear calibration relationships. The continued use of any chromatographic procedure requires a verification of the calibration relationship, and procedures for such verifications are described in this method as well (see Sec. 7.7).

2.6 The identification of target compounds by any chromatographic procedure is based, at least in part, on retention times. Sec. 7.6 provides procedures for the determination of retention times and retention time windows to be used with the specific methods listed in Sec. 1.1.

2.7 The calculations necessary to derive sample-specific concentration results from the instrument responses are common to most of the analytical methods listed in Sec. 1.1. Therefore, Sec. 7.10 of Method 8000 contains a summary of the commonly used calculations.

2.8 Preventive maintenance and corrective actions are essential to the generation of quality data in a routine laboratory setting. Suggestions for such procedures are found in Sec. 7.11.

2.9 Most of the methods listed in Sec. 1.1 employ a common approach to quality control (QC). While some of the overall procedures are described in Chapter One, Sec. 8.0 describes routinely used procedures for calibration verification, instrument performance checks, demonstrating acceptable performance, etc.

2.10 Before performing analyses of specific samples, analysts should determine acceptable recovery ranges for all target analytes of interest in the type of matrices to be tested. These procedures are described in Secs. 8.4, 8.5, and 8.7. Analysts must also be able to demonstrate that the sensitivity of the procedure employed is appropriate for the intended application. One approach to such a demonstration is to estimate the method detection limits for the analytes of interest using the procedures in Chapter One.

3.0 INTERFERENCES/CHROMATOGRAPHIC PERFORMANCE

3.1 Contamination by carryover can occur whenever high-concentration and low-concentration samples are analyzed in sequence. To reduce the potential for carryover, the sample syringe or purging device must be thoroughly rinsed between samples with an appropriate solvent. Purge and trap devices or headspace devices should be thoroughly baked out between samples. Where practical, samples with unusually high concentrations of analytes should be followed by a solvent blank or by an analysis of organic-free reagent water to check for cross-contamination. If the target compounds present in an unusually concentrated sample are also found to be present in the subsequent samples, the analyst must demonstrate that the compounds are not due to carryover. Conversely, if those target compounds are <u>not</u> present in the subsequent sample, then the analysis of a solvent blank or organic-free reagent water is not necessary.

Purging vessels may be cleaned by rinsing with methanol, followed by a distilled water rinse and drying in a 105°C oven between analyses. Detergent solutions may also be used, but care must be taken to remove the detergent residue from the purging vessel. Other approaches to cleaning purging vessels may also be employed, provided that the laboratory can demonstrate that they are effective in removing contaminants.

3.2 In addition to carryover of compounds from one sample to the next, the analysis of high-concentration samples can lead to contamination of the analytical instrument itself. This is particularly true for GC/MS. Eliminating this contamination can require significant time and effort in cleaning the instruments, time that cannot be spent analyzing samples. The most reliable procedure for ensuring minimum down time during the GC/MS analysis of samples is to screen samples by some other technique. Samples to be analyzed for volatiles can be screened using an automated headspace sampler (Method 5021) connected to a GC/PID/ELCD detector (Method 8021). Samples to be analyzed for semivolatiles can be screened using GC/FID. Other screening methods are also acceptable. The analyst should use the screening results to choose an appropriate dilution factor for the GC/MS analysis that will prevent system contamination yet still provide adequate sensitivity for the major constituents of the sample.

3.3 One of the most important measures of chromatographic performance is resolution, the separation of chromatographic peaks (peak separation/average peak width). Peak separations are facilitated by good column efficiency (i.e., narrow peak widths) and good column selectivity (i.e., analytes partition differently between the mobile and stationary phases).

3.3.1 The goal of analytical chromatography is to separate sample constituents within a reasonable time. Baseline resolution of each target analyte from co-extracted materials provides the best quantitative results, but is not always possible to achieve.

3.3.2 In general, capillary columns contain a greater number of theoretical plates than packed columns. (A theoretical plate is a surface at which an interaction between the sample components and the stationary phase may occur). As a result, capillary columns generally provide more complete separation of the analytes of interest. However, packed columns can provide adequate resolution of some analytes and are most appropriately employed when the list of analytes to be determined is relatively short.

3.3.3 The ability to resolve individual compounds is generally the limiting factor for the number of analytes that can be measured using a single procedure. Some procedures, particularly Method 8081 (Organochlorine Pesticides), Method 8082 (PCBs), and Method 8141 (Organophosphorus Pesticides), list analytes that may not all be resolved from one another. Therefore, while each of these methods is suitable for the listed compounds, they may not be suitable to measure the entire list in a single analysis. In addition, some methods include analytes that are isomers or closely related compounds which are well-known as co-eluting or are not completely separable. In these instances, the results should be reported as the sum of the two (or more) analytes. Laboratories should demonstrate that target analytes are resolved during calibration and satisfy the requirements in Sec. 8.3, or should report the results as "totals" or "sums" (e.g., m+p-xylene). Methods that utilize mass spectrometry for detection are less affected by resolution problems, because overlapping peaks may often be mass-resolved. However, even mass spectrometry will not be able to mass resolve positional isomers such as m-xylene and p-xylene if the compounds co-elute.

3.4 Elevated chromatographic baselines should be minimized or eliminated during these analyses. Baseline humps can usually be reduced or eliminated by the application of appropriate sample clean-up (see Method 3600), extract dilution, the use of pre-columns and/or inserts, or use of a selective detector. Integration of "hump-o-grams" can result in significant quantitative errors. When elevated baselines are observed during the analysis of blanks and standards, the chromatographic system should be considered contaminated. This contamination may be the result of impure carrier gas, inadequate gas conditioning, septum bleed, column oxidation, and/or pyrolysis products in the injector or column. Such contamination is unacceptable and should be addressed through a program of preventive maintenance and corrective action.

3.5 GC preventive maintenance and corrective action

Poor GC performance may be expected whenever a chromatographic system is contaminated with high-boiling materials, particularly in the injector. Analysts should perform routine maintenance, including replacement of septa, cleaning and deactivating injector liners, and removing as much as 0.5 - 1 m from the injector side of a capillary column.

If chromatographic performance or ghost peaks are still a problem, cleaning of the metallic surfaces of the injection port itself may be necessary. Capillary columns are reliable and easy to use, but several specific actions are necessary to ensure good performance.

3.5.1 Contact between the capillary column and the wall of the GC oven can affect both chromatographic performance and column life. Care should be taken to prevent the column from touching the oven walls.

3.5.2 Care should be taken to keep oxygen out of capillary columns.

3.5.3 Septa should only be changed after the oven has cooled.

3.5.4 Columns should be flushed with carrier gas for 10 minutes before reheating the oven.

3.5.5 Carrier gas should be scrubbed to remove traces of oxygen and scrubbers should be changed regularly.

3.5.6 Carrier gas should always be passed through the column whenever the oven is heated.

3.6 HPLC preventive maintenance and corrective action

HPLC band broadening results from improper instrument setup or maintenance. Band broadening results whenever there is a dead volume between the injector and the detector. Therefore, plumbing connections should be of minimum length and diameter, and ferrules should be properly positioned on the tubing to minimize dead volume.

3.6.1 Columns should not be subjected to sudden physical stress (e.g., dropping) or solvent shocks (e.g., changing solvents without a gradient).

3.6.2 Columns can become contaminated with particulates or insoluble materials. Guard columns should be used when dirty samples are analyzed.

3.6.3 High quality columns are packed uniformly with small uniform diameter particles with a minimum number of free silol groups. Use of such columns will result in optimum chromatographic performance.

3.6.4 Columns should be replaced when performance degrades (e.g., significant band broadening, peak splitting, or loss of chromatographic resolution occurs).

3.6.5 Pumping systems should deliver reproducible gradients at a uniform flow rate. Rates can be checked by collecting solvent into a graduated cylinder.

3.6.6 Column temperatures should be regulated by the use of column temperature control ovens to ensure reproducibility of retention times.

3.6.7 Small changes in the composition or pH of the mobile phase can have a significant effect on retention times.

4.0 APPARATUS AND MATERIALS

4.1 GC inlet systems

4.1.1 Volatile organics

Volatile organic analytes are introduced into a GC through a purge-and-trap system, by direct injection, or by other devices. The purge-and-trap apparatus is described in Method 5030 for water samples and in Method 5035 for soil and other solid samples. See Method 5000 for guidance on all forms of sample introduction of volatiles into the GC and GC/MS system.

4.1.2 Semivolatile organics

Sample extracts containing semivolatile organic compounds are introduced into a GC with a syringe that passes through a septum into an injection port. The injection port allows the sample extract to be vaporized prior to being flushed onto the GC column, hence the term "gas" chromatography. Correct set up and maintenance of the injector port is necessary to achieve acceptable performance with GC methods. Septa should be changed frequently enough to prevent retention time shifts of target analytes and peak tailing. The schedule for such septa changes is dependent on the quality of the septa, the sharpness of the needle, and the operation of the injection system. Appropriate injector liners should be installed, and liners should be cleaned and deactivated (with dichlorodimethylsilane) regularly.

4.1.3 Injector difficulties include the destruction of labile analytes and discrimination against high boiling compounds in capillary injectors.

4.1.3.1 Packed columns and wide-bore capillary columns (> 0.50 mm ID) should be mounted in 1/4-inch injectors. An injector liner is needed for capillary columns.

4.1.3.2 Narrow-bore capillary columns (≤ 0.32 mm ID) should be mounted in split/splitless (Grob-type) injectors. Split/splitless injectors require automated valve closures that direct most of the flow (and sample) onto the head of the analytical column. After 30 - 45 seconds, the split valve is opened, so that most of the flow is vented during analysis, thus eliminating the solvent tail, and maintaining proper flow through the column. The initial oven temperature should be below the boiling point of the injection solvent if the solvent front interferes with early eluting analytes or if the solvent effect is needed to resolve difficult-to-separate analytes.

4.1.3.3 Cool on-column injection allows the analysis of labile compounds that degrade on packed columns and in split/splitless injectors.

4.2 GC flow control

Precise control of the gas mobile phase is necessary to achieve reproducible GC retention times. Flow controllers within any GC used for SW-846 analyses must deliver a precisely metered gas flow at a rate appropriate for the GC column mounted in the instrument.

4.2.1 Most GCs have restrictors built into flow controllers. These restrictors are used to provide precise flow at the carrier gas flow rate specified in the method (e.g., use <20 mL/min restrictors for wide-bore capillary methods). Carrier gas flow rates should be checked regularly (with both the injector and the oven heated) using a bubble meter or other appropriate procedure.

4.2.2 Cylinder pressures should also be regulated properly. Manifold pressures must be sufficiently large that a change in the head pressure of an individual instrument does not affect the flow through all instruments. Toggle valves that allow instruments to be isolated are recommended for all multi-instrument gas delivery systems. Analysts should spend time each week conducting preventative maintenance in order to ensure that proper flow control is maintained. One needs to search for leaks using a helium tester or soap solution at each connector in the gas delivery systems. Analysts should routinely conduct preventive maintenance activities, including those designed to ensure proper flow control and to identify potential leaks in the gas delivery system. The search for leaks may be conducted with a helium leak tester, soap solutions, performing static pressure tests, or other appropriate measures.

4.2.3 Carrier gas should be of high purity and should be conditioned between the cylinder and the GC to remove traces of water and oxygen. Scrubbers should be changed according to manufacturers recommendations. Gas regulators should contain stainless steel diaphragms. Neoprene diaphragms are a potential source of gas contamination, and should not be used.

4.3 Gas chromatographic columns

Each determinative method in SW-846 provides a description of a chromatographic column or columns with associated performance data. Other packed or capillary (open-tubular) columns may be substituted in SW-846 methods to improve performance if (1) the requirements of Secs. 8.3 and 8.4 are satisfied, and (2) target analytes are sufficiently resolved from one another and from co-extracted interferences to provide data of the appropriate quality for the intended application.

4.3.1 Narrower columns are more efficient (i.e., can resolve more analytes) but have a lower capacity (i.e., can accept less sample without peak distortion).

4.3.2 Longer columns can resolve more analytes, as resolution increases as a function of the square root of column length.

4.3.3 Increasing column film thickness or column loading increases column capacity and retention times.

4.3.4 Use of capillary columns has become standard practice in environmental and waste analysis. Capillary columns have an inherently greater ability to separate analytes than packed columns. However, packed columns can provide adequate resolution of some analytes and are most appropriately employed when the list of analytes to be determined is relatively short.

4.3.5 Columns used for SW-846 analyses should be installed properly. Column ends should be cut square. Contaminated ends should be trimmed off, and columns should be placed through ferrules before they are trimmed. Columns should not touch the walls of the GC oven during analysis, and the manufacturer's column temperature limits should not be exceeded.

4.3.6 Septa should be changed regularly and septum nuts should not be overtightened. Oxygen should not be introduced into a hot column and carrier gas should be passed through a column whenever it is heated. New columns, particularly packed columns, should be conditioned prior to analyzing samples.

4.4 GC detectors

Detectors are the transducers that respond to components that elute from a GC column and produce the electrical signal that is used for quantitative determinations. SW-846 analyses are conducted using selective detectors or mass spectrometers listed in Sec. 1.1. Except where otherwise recommended by the instrument manufacturer, selective non-MS detectors should be maintained at least 20°C above the highest oven temperature employed to prevent condensation and detector contamination. The transfer lines between the GC and an MS detector should be maintained at a temperature above the highest column temperature, or as specified by the instrument manufacturer, to prevent condensation.

4.5 HPLC injectors

Liquids are essentially non-compressible, so a mechanical device is necessary that allows introduction of the sample into a high pressure flow without significant disruption in the flow rate and hydraulic pressure. Normally, a 6-port valve is used for this purpose. A sample loop (generally 10-100 µL) is isolated from the flow of the mobile phase and filled with a sample extract. (Larger sample loops may be used to increase sensitivity, however, they may degrade chromatographic performance). The extract is then injected by turning the valve so that the mobile phase flows through the loop. This procedure virtually eliminates dead volume in the injector and is fully compatible with automated operation.

4.5.1 When the extract is highly viscous, a pressure spike results which can automatically shut off the HPLC pump.

4.5.2 Contamination of subsequent injections may occur when the extract contains material that is not soluble in the mobile phase.

4.5.3 Injection loops are easily changed but analysts must ensure that the compression fittings are properly installed to prevent leaks. Injectors require maintenance, as the surfaces that turn past each other do wear down.

4.6 HPLC pumps

The mobile phase used for HPLC must be accurately pressurized before it enters the injector. HPLC pumps are generally capable of delivering solvent at 5000 psi with excellent precision. The rate of delivery depends on the column that is used for the separation. Most environmental methods recommend flow rates of 0.25-1.0 mL/min. Flow rates should be checked by collecting column effluent in a graduated cylinder.

Most pumping systems are capable of changing solvent concentration during an analysis (i.e., gradient elution). Gradients are generated by either high pressure mixing of two streams between the pump and the injector or by proportional mixing of the solvents before they are pumped. In either case, solvent mixing can cause changes in the solubility of dissolved gases, the formation of bubbles in the mobile phase, or non-reproducible gradients.

4.6.1 Air bubbles result in erratic baseline and, in the case of low pressure mixing, bubbles can cause the pump to cavitate. Therefore, HPLC solvents should be degassed prior to use.

4.6.2 Non-reproducible gradients can result in significant changes in retention times from run to run.

4.6.3 HPLC solvents should be filtered to remove particles that cause pump piston wear. HPLC pump maintenance includes replacing seals regularly. (Use of strong buffers or solvents like tetrahydrofuran can significantly shorten the lifetime of pump seals.) Pumps should deliver solvent with minimal pulsation.

4.7 HPLC Columns

These columns must be constructed with minimum dead volume and a narrow particle size distribution. HPLC columns are generally constructed of stainless steel tubing and are sealed with compression fittings. Manufacturers provide columns that are bonded with different alkyl groups (e.g., C_{18}, cyano, TMS), have different percent carbon loading, are packed with different particle sizes (3-10 µm), and are packed with particles of different pore size (smaller pores mean greater surface area), or are of different dimensions.

4.7.1 Columns with higher percent loading have the capacity to analyze somewhat larger samples, but extremely high loadings may contribute to problems with the particle beam MS interface.

4.7.2 Columns with free silol groups show less tailing of polar materials (e.g., amines).

4.7.3 A smaller particle (and pore) size generally gives better resolution, higher back pressure, and smaller sample capacity. Columns with 3 µm particle size may have short lifetimes when they are used for the analysis of complex waste extracts.

4.7.4 Improvements in column packing have resulted in 10 and 15 cm columns that provide the separating power necessary for most environmental and waste analyses.

4.7.5 Internal diameters of columns used for environmental and waste analysis are generally 2-5 mm. Narrower columns are called microbore columns. While they provide better separations, they become fouled more easily.

4.7.6 The lifetime and performance of HPLC columns can be improved through proper maintenance. Analysts should filter sample extracts, use compatible guard columns, check for clogged frits and for column voids. Columns should not be stored dry or containing strong buffers.

4.8 HPLC column temperature control ovens

HPLC retention times are much more reproducible if the column is held at a constant temperature. Temperature control ovens capable of maintaining the HPLC column at ± 0.1°C should be utilized to maintain consistent retention times throughout the course of an HPLC analysis. Normal oven operating temperature would be 3-5°C above ambient laboratory temperature.

4.9 HPLC detectors

Detectors are the transducers that respond to components that elute from a HPLC column and produce the electrical signal that is used for quantitative determinations. SW-846 analyses are conducted using selective detectors or mass spectrometers listed in Sec. 1.1. HPLC/MS requires the use of a sophisticated interface that separates target analytes from the aqueous mobile phase. Examples include the thermospray (TSP), electrospray (ESP), and the particle beam (PB) interfaces.

4.10 Data systems

Raw chromatographic data have to be reduced in order to provide the quantitative information required by analysts. The use of sophisticated data systems is strongly recommended for SW-846 chromatographic methods. The ability to store and replot chromatographic data is invaluable during data reduction and review. Organizations should establish their priorities and select the system that is most suitable for their applications.

4.11 Supplies

Chromatographers require a variety of supplies. The specific items that should be stocked depend on laboratory instrumentation and the analyses performed. At a minimum, laboratories need PTFE tape, stainless steel regulators, acid-washed copper tubing, and syringes, and replacement parts for instruments.

4.11.1 Laboratories performing GC analyses also require high purity gases, scrubbers for gas conditioning, gas-tight fittings, capillary cutters, magnifying glasses, septa with proper temperature limits, appropriate ferrules, dichlorodimethylsilane (for deactivating surfaces), glass wool, spare columns, and injection port liners.

4.11.2 Laboratories performing HPLC analyses require high purity solvents, column packing material, frits, 1/16-inch tubing, appropriate ferrules, solvent filtration apparatus, and solvent degassing apparatus.

5.0 REAGENTS

See the specific extraction and determinative methods for the reagents needed.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

Refer to Chapter Four, Organic Analytes, Sec. 4.1, for information on sample collection, preservation and handling procedures. Additional information may be found in some of the individual sample extraction, preparation, and determinative methods.

7.0 PROCEDURE

Extraction and cleanup are critical for the successful analyses of environmental samples and wastes. Analysts should pay particular attention to selection of sample preparation procedures to obtain reliable measurements.

7.1 Extraction

The individual determinative methods for organic analytes in SW-846 often recommend appropriate sample extraction procedures. General guidance on semivolatile extraction procedures can be found in Method 3500. Guidance on volatile procedures can be found in Method 5000.

7.2 Cleanup and separation

The individual determinative methods for organic analytes in SW-846 often recommend appropriate cleanup procedures. General guidance on cleanup procedures can be found in Method 3600. While some relatively clean matrices (such as ground water samples) may not require extensive cleanups, the analyst should carefully balance the time savings gained by skipping cleanups against the potential increases in instrument down time and loss of data quality that can occur as a result.

7.3 Recommended chromatographic columns and instrument conditions are described in each determinative method. As noted earlier, these columns and conditions are typically those used during the development and testing of the method. However, other chromatographic systems may have somewhat different characteristics. In addition, analytical instrumentation continues to evolve. Therefore, SW-846 methods allow analysts some flexibility to change these conditions (with certain exceptions), as long as they demonstrate adequate performance.

Chromatographic performance is demonstrated by the resolution of standards and the ability to model the response of the detector during calibration, and by the sensitivity, accuracy, precision, frequency of false positives, and frequency of false negatives during analysis. If the laboratory employs an alternative chromatographic procedure or alternative conditions, then the laboratory must demonstrate that the performance satisfies the analytical requirements of the specific application for which the alternative chromatographic procedure is being used. Such demonstrations should be performed using the procedures outlined in Secs. 8.2 to 8.5 of this method and those in Chapter One.

7.4 Initial Calibration

Calibration of an analytical instrument involves the delineation of the relationship between the response of the instrument and the amount or concentration of an analyte introduced into the instrument. The graphical depiction of this relationship is often referred to as the calibration curve. In order to perform quantitative measurements, this relationship must be established prior to the analysis of any samples, and thus, is termed initial calibration.

Historically, many analytical methods have relied on linear models of the calibration relationship, where the instrument response is directly proportional to the amount of a target compound. The linear model has many advantages, among them, simplicity and ease of use. Unfortunately, given the advent of new detection techniques and the fact that many techniques cannot be optimized for all of the analytes to which they may be applied, the analyst is increasingly likely to encounter situations where the linear model neither applies nor is appropriate.

The initial calibration for SW-846 chromatographic methods involves the analysis of standards containing the target compounds at a minimum of five different concentrations covering the working range of the instrument. In order to produce acceptable sample results, the response of the instrument must be within the working range established by the initial calibration. The extrapolation of the calibration to concentrations above or below those of the actual calibration standards is <u>not</u> appropriate and may lead to significant quantitative errors regardless of the calibration model chosen. Analysts are advised that it may be necessary to prepare calibration standards that cover concentration ranges that are appropriate for specific projects or type of analyses. For instance, the analyst should not necessarily expect to be able to perform a calibration appropriate for sub-ppb level analyses and also use the same calibration data for high-ppb or ppm level samples.

The specific options for evaluating the initial calibration are described in Sec. 7.5. The remainder of this section describes the preparation of calibration standards, the use of external and internal standard calibrations, and the calculation of both calibration factors and response factors.

7.4.1 Calibration standards are prepared using the procedures indicated in Sec. 5.0 of the determinative method of interest. However, the general procedure is described here.

7.4.1.1 For each analyte and surrogate of interest, prepare calibration standards at a minimum of five different concentrations by adding volumes of one or more stock standards to volumetric flasks and diluting to volume with an appropriate solvent.

7.4.1.2 The lowest concentration calibration standard that is analyzed during an initial calibration establishes the method quantitation limit based on the final volume of extract (or sample) described in the preparative method or employed by the laboratory.

7.4.1.3 The other concentrations should define the working range of the detector or correspond to the expected range of concentrations found in actual samples that are also within the working range of the detector.

7.4.1.4 For each analyte, at least one of the calibration standards should correspond to a sample concentration at or below that necessary to meet the data quality objectives of the project, which may include establishing compliance with a regulatory or action limit.

7.4.1.5 Given the number of target compounds addressed by some of the methods listed in Sec. 1.1, it may be necessary to prepare several sets of calibration standards, each set consisting of five solutions at different concentrations. The initial calibration will then involve the analysis of each of these sets of five standards.

7.4.1.6 Once the standards have been prepared, the initial calibration begins by establishing chromatographic operating parameters that provide instrument performance equivalent to that documented in Sec. 7.0 of the determinative method of interest, or that is appropriate for the data quality objectives of the intended application.

7.4.2 External standard and internal standard calibration techniques

The chromatographic system may be calibrated using either the external standard or the internal standard techniques described below. General calibration criteria are provided in this section for GC and HPLC procedures using non-MS detection. The applicable calibration procedures for GC/MS (e.g., Methods 8260, 8270, 8280, and 8290), HPLC/MS (e.g., Methods 8321 and 8325), and GC/FT-IR (e.g., Method 8410) are described in those methods. Some

determinative methods may provide special guidance on calibration that is specific to that method.

Regardless of whether external standard or internal standard calibration is used, introduce each calibration standard into the instrument using the same technique that is used to introduce the actual samples into the gas chromatograph (e.g., 1-3 µL injections for GC methods, 10-100 µL injections for HPLC methods, purge-and-trap techniques for volatiles, etc.). Tabulate peak area or height responses against the mass or concentration injected, as described below.

7.4.2.1 External standard calibration procedure

External standard calibration involves comparison of instrument responses from the sample to the responses from the target compounds in the calibration standards. Sample peak areas (or peak heights) are compared to peak areas (or heights) of the standards. The ratio of the detector response to the amount (mass) of analyte in the calibration standard is defined as the calibration factor (CF).

$$CF = \frac{\text{Peak Area (or Height) of the Compound in the Standard}}{\text{Mass of the Compound Injected (in nanograms)}}$$

For multi-component analytes, see the appropriate determinative method for information on which areas to employ.

The CF can also be calculated using the concentration of the standard rather than the mass in the denominator of the equation above. However, the use of concentrations in CFs will require changes to the equations that are used to calculate sample concentrations (see Sec. 7.10.1.1).

7.4.2.2 Internal standard calibration procedure

Internal standard calibration involves the comparison of instrument responses from the target compounds in the sample to the responses of specific standards added to the sample or sample extract prior to injection. The ratio of the peak area (or height) of the target compound in the sample or sample extract to the peak area (or height) of the internal standard in the sample or sample extract is compared to a similar ratio derived for each calibration standard. The ratio is termed the response factor (RF), and may also be known as a relative response factor in other methods.

In many cases, internal standards are recommended in SW-846 methods. These recommended internal standards are often brominated, fluorinated, or stable isotopically labeled analogs of specific target compounds, or are closely related compounds whose presence in environmental samples is highly unlikely. If internal standards are not recommended in the method, then the analyst needs to select one or more internal standards that are similar in analytical behavior to the compounds of interest, and not expected to be found in the samples otherwise.

Whichever internal standards are employed, the analyst needs to demonstrate that the measurement of the internal standard is not affected by method analytes and surrogates or by matrix interferences. In general, internal standard calibration is not as useful for GC and HPLC methods with non-MS detectors because of the inability to chromatographically resolve many internal standards from the target compounds. The

use of MS detectors makes internal standard calibration practical because the masses of the internal standards can be resolved from those of the target compounds even when chromatographic resolution cannot be achieved.

When preparing calibration standards for use with internal standard calibration, add the same amount of the internal standard solution to each calibration standard, such that the concentration of each internal standard is constant across all of the calibration standards, whereas the concentrations of the target analytes will vary. The internal standard solution will contain one or more internal standards and the concentration of the individual internal standards may differ within the spiking solution (e.g., not all internal standards need to be at the same concentration in this solution). The mass of each internal standard added to each sample extract immediately prior to injection into the instrument or to each sample prior to purging must be the same as the mass of the internal standard in each calibration standard. The volume of the solution spiked into sample extracts should be such that minimal dilution of the extract occurs (e.g., 10 µL of solution added to a 1 mL final extract results in only a negligible 0.1% change in the final extract volume which can be ignored in the calculations).

An ideal internal standard concentration would yield a response factor of 1 for each analyte. However, this is not practical when dealing with more than a few target analytes. Therefore, as a general rule, the amount of internal standard should produce an instrument response (e.g., area counts) that is no more than 100 times that produced by the lowest concentration of the least responsive target analyte associated with the internal standard. This should result in a minimum response factor of approximately 0.01 for the least responsive target compound.

For each of the initial calibration standards, calculate the RF values for each target compound relative to one of the internal standards as follows:

$$RF = \frac{A_s \times C_{is}}{A_{is} \times C_s}$$

where:

A_s = Peak area (or height) of the analyte or surrogate.
A_{is} = Peak area (or height) of the internal standard.
C_s = Concentration of the analyte or surrogate, in µg/L.
C_{is} = Concentration of the internal standard, in µg/L.

Note that in the equation above, RF is unitless, i.e., the units from the two area terms and the two concentration terms cancel out. Therefore, units other than µg/L may be used for the concentrations of the analyte, surrogate, and internal standard, provided that both C_s and C_{is} are expressed in the same units. The mass of the analyte and internal standard may also be used in calculating the RF value.

7.5 Calibration linearity

SW-846 chromatographic methods allow the use of both linear and non-linear models for the calibration data, as described below. Given the limitations in instrument data systems, it is likely that the analyst will have to choose one model for all analytes in a particular method. Both models can be applied to either external or internal standard calibration data.

NOTE: The option for non-linear calibration may be necessary to achieve low detection limits or to address specific instrumental techniques. However, it is not EPA's intent to allow non-linear calibration to be used to compensate for detector saturation at higher concentrations or to avoid proper instrument maintenance.

Whichever calibration model is employed, a unique analyte or surrogate concentration must fall within the calibration range. Samples with concentrations that exceed the calibration range must be diluted to fall within the range.

NOTE: The following sections describe various options for initial calibration and provide the calibration acceptance criteria used to evaluate each option. The criteria listed in these sections are designed for quantitation of trace level concentrations of the analytes of interest. If data of lesser quality will satisfy project-specific data needs, then less stringent criteria may be employed, provided that they are documented and approved in a project-specific QA project plan.

The choice of a specific calibration model should be made in one of two ways. The first is to begin with the simplest approach, the linear model through the origin, and progressing through the other options until the calibration acceptance criteria are met. The second approach is to use *a priori* knowledge of the detector response to choose the calibration model. Such knowledge may come from previous experience, knowledge of the physics of the detector, or specific manufacturer's recommendations.

7.5.1 Linear calibration using the average calibration or response factor

When calculated as described in Sec. 7.4, both calibration factors and response factors are a measure of the slope of the calibration relationship and assume that the curve passes through the origin. Under ideal conditions, the factors will not vary with the concentration of the standard that is injected into the instrument. In practice, some variation is to be expected. However, when the variation, measured as the relative standard deviation (RSD), is less than or equal to 20%, the use of the linear model is generally appropriate, and the calibration curve can be assumed to be linear and to pass through the origin.

NOTE: Linearity through zero is a statistical assumption and not a rationale for reporting results below the calibration range demonstrated by the analysis of the standards

To evaluate the linearity of the initial calibration, calculate the mean CF (external standard calibration) or RF (internal standard calibration), the standard deviation (SD), and the RSD as follows:

$$\text{mean CF} = \overline{CF} = \frac{\sum_{i=1}^{n} CF_i}{n} \qquad \text{mean RF} = \overline{RF} = \frac{\sum_{i=1}^{n} RF_i}{n}$$

$$SD = \sqrt{\frac{\sum_{i=1}^{n} (CF_i - \overline{CF})^2}{n-1}} \qquad SD = \sqrt{\frac{\sum_{i=1}^{n} (RF_i - \overline{RF})^2}{n-1}}$$

$$RSD = \frac{SD}{\overline{CF}} \times 100 \qquad RSD = \frac{SD}{\overline{RF}} \times 100$$

where *n* is the number of calibration standards and RSD is expressed as a percentage (%).

If the RSD of the calibration or response factors is less than or equal to 20% over the calibration range, then linearity through the origin may be assumed, and the average calibration or response factor may be used to determine sample concentrations.

7.5.1.1 Given the potentially large numbers of analytes that may be analyzed in some methods, it is likely that some analytes may exceed the 20% acceptance limit for the RSD for a given calibration. In those instances, the following steps are recommended, but not required.

The first step is generally to check the instrument operating conditions. The suggested maintenance procedures in Sec. 7.11 may be useful in guiding such adjustments. This option will apply in those instances where a linear instrument response is expected. It may involve some trade-offs to optimize performance across all target analytes. For instance, changes to the operating conditions necessary to achieve linearity for problem compounds may cause the RSD for other compounds to increase, but as long as all analytes meet the RSD limits for linearity, the calibration is acceptable.

If the RSD for any analyte is greater than 20%, the analyst may wish to review the results (area counts, calibration or response factors, and RSD) for those analytes to ensure that the problem is not associated with just one of the five initial calibration standards. If the problem appears to be associated with a single standard, that one standard may be reanalyzed and the RSD recalculated. Replacing the standard may be necessary in some cases.

A third alternative is to narrow the calibration range by replacing one or more of the calibration standards with standards that cover a narrower range. If linearity can be achieved using a narrower calibration range, document the calibration linearity, and proceed with analyses. The changes to the upper end of the calibration range will affect the need to dilute samples above the range, while changes to the lower end will affect the overall sensitivity of the method. Consider the regulatory limits or action levels associated with the target analytes when adjusting the lower end of the range.

NOTE: As noted in Sec. 7.4.1.2, the method quantitation limit is established by the concentration of the lowest standard analyzed during the initial calibration. Hence, narrowing the calibration range by changing the concentration of the lowest standard will, by definition, change the method quantitation limit. When the purpose of the analysis is to demonstrate compliance with a specific regulatory limit or action level, the analyst must ensure that the method quantitation limit is at least as low as the regulatory limit or action level.

7.5.1.2 In those instances where the RSD for one or more analytes exceeds 20%, the initial calibration may still be acceptable if the following conditions are met:

7.5.1.2.1 The mean of the RSD values for all analytes in the calibration is less than or equal to 20%. The mean RSD is calculated by summing the RSD value for each analyte and dividing by the total number of analytes. If no analyte has an RSD above 20%, then the mean RSD calculation need not be performed.

7.5.1.2.2 The mean RSD criterion applies to all analytes in the standards, regardless of whether or not they are of interest for a specific project. In other words, if the target analyte is part of the calibration standard, its RSD value is included in the evaluation.

7.5.1.2.3 The data user must be provided with either a summary of the initial calibration data or a specific list of those compounds for which the RSD exceeded 20% and the results of the mean RSD calculation.

NOTE: The analyst and the data user must be aware that the use of the approach listed in Sec. 7.5.1.2.1 (i.e., the average of all RSD values ≤ 20%) will lead to greater uncertainty for those analytes for which the RSD is greater than 20%. The analyst and the data user should review the associated quality control results carefully, with particular attention to the matrix spike and laboratory control sample results (see Sec. 8.0), to determine if the calibration linearity poses a significant concern. If this approach is not acceptable for a particular application, then the analyst may need to employ one of the other calibration approaches (see Secs. 7.5.2 to 7.5.4) or adjust the instrument operating conditions and/or the calibration range until the RSD is ≤ 20%.

7.5.1.3 If all of the conditions in Sec. 7.5.1.2 are met, then the average calibration or response factor may be used to determine sample concentrations, as described in Sec. 7.10.

7.5.2 Linear calibration using a least squares regression

If the RSD of the calibration or response factors is greater than 20% over the calibration range, then linearity through the origin cannot be assumed. If this is the case, the analyst may employ a regression equation that does not pass through the origin. This approach may also be employed based on past experience or *a priori* knowledge of the instrument response. Further, at the discretion of the analyst, this approach also may be used for analytes that do meet the RSD limits in Sec. 7.5.1.

This is most easily achieved by performing a linear regression of the instrument response versus the concentration of the standards. Make certain that the instrument response is treated as the dependent variable (y) and the concentration as the independent variable (x). This is a statistical requirement and is not simply a graphical convention.

The analyst may also employ a weighted least squares regression if replicate multi-point calibrations have been performed, e.g., three 5-point curves. For all other instances, an appropriate unweighted least squares method should be used. When using a weighted linear least squares regression, the following weighting factor should be used:

$$\frac{1}{SD^2}$$

where SD is the standard deviation of the replicate results at each individual standard concentration.

The regression will produce the slope and intercept terms for a linear equation in the form:

$$y = ax + b$$

where:

y = Instrument response (peak area or height)
a = Slope of the line (also called the coefficient of x)
x = Concentration of the calibration standard
b = The intercept

The analyst should not force the line through the origin, but have the intercept calculated from the five data points. Otherwise, the problems noted with the RSD value will occur, i.e., a line through the origin will not meet the QC specifications. In addition, do not include the origin (0,0) as a sixth calibration point. The use of a linear regression may not be used as a rationale for reporting results below the calibration range demonstrated by the analysis of the standards. The regression calculation will generate a correlation coefficient (r) that is a measure of the "goodness of fit" of the regression line to the data. A value of 1.00 indicates a perfect fit. In order to be used for quantitative purposes, r must be greater than or equal to 0.99.

In calculating sample concentrations by the external standard method, the regression equation is rearranged to solve for the concentration (x), as shown below.

$$x = \frac{(y - b)}{a}$$

When a weighted linear least squares regression is employed, the regression equation becomes:

$$y = \frac{1}{SD^2}(ax + b)$$

which may be rearranged to solve for x, the concentration. Using internal standard quantitation, the regression equation is rearranged as shown below:

$$\frac{A_s C_{is}}{A_{is}} = aC_s + b$$

where:

A_s = Area (or height) of the peak for the target analyte in the sample
A_{is} = Area (or height) of the peak for the internal standard
C_s = Concentration of the target analyte in the calibration standard
C_{is} = Concentration of the internal standard
a = Slope of the line (also called the coefficient of C_s)
b = The intercept

In calculating sample concentrations by the internal standard method, the regression equation is rearranged to solve for the concentration of the target analyte (C_s), as shown below.

$$C_s = \frac{\left[\frac{A_s C_{is}}{A_{is}} - b\right]}{a}$$

7.5.3 Non-linear calibration

In situations where the analyst knows that the instrument response does not follow a linear model over a sufficiently wide working range, or when the other approaches described here have not met the acceptance criteria, a non-linear calibration model may be employed.

NOTE: It is not EPA's intent to allow non-linear calibration to be used to compensate for detector saturation at higher concentrations or to avoid proper instrument maintenance. Thus, non-linear calibration should not be employed for methods or instruments previously shown to exhibit linear calibration for the analytes of interest.

When using a calibration model for quantitation, the curve must be continuous, continuously differentiable and monotonic over the calibration range. The model chosen should have no more than four parameters, i.e., if the model is polynomial, it may be no more than third order, as in the equation:

$$y = ax^3 + bx^2 + cx + d$$

As noted above, the model must be continuous. A curve is continuous when it has consecutive numerical values along the function, whether increasing or decreasing, and without having breaks in the function (i.e., the pen shall never leave the paper from the minimum to the maximum). The model must also be continuously differentiable, such that all derivatives of the function are continuous functions themselves, and monotonic, such that all tangent lines of the derivative to all of the points on the calibration curve have either only positive or negative slopes.

If the model is not a polynomial, it should not include more than four parameters, i.e.,

$$y = f(a,b,c,d,x)$$

where "f" indicates a function with up to four parameters.

In estimating model parameters for the calibration data, the instrumental response (y) must be treated as the dependent variable, and the calibration of the concentration standard (x) must be the independent variable. Do not force the line through the origin, i.e., do not set the intercept as 0, and do not include the origin (0,0) as a calibration point. Model estimates from the regression must be used as calculated, i.e., if the model is a polynomial, the intercept is d and may not be set to 0. Weighting in a calibration model may significantly improve its accuracy.

The statistical considerations in developing a non-linear calibration model require more data than the more traditional linear approaches described above. Whereas SW-846 methods employ five standards for a linear (first order) calibration model, a quadratic (second order) model requires six standards, and a third order polynomial requires seven standards.

Most curve fitting programs will use some form of least squares minimization to adjust the coefficients of the polynomial (a,b,c, and d, above) to obtain the polynomial that best fits the data. The "goodness of fit" of the polynomial equation is evaluated by calculating the weighted coefficient of the determination (COD).

$$\mathrm{COD} = \frac{\sum_{i=1}^{n}(y_{obs} - \bar{y})^2 - \left(\frac{n-1}{n-p}\right)\sum_{i=1}^{n}(y_{obs} - Y_i)^2}{\sum_{i=1}^{n}(y_{obs} - \bar{y})^2}$$

where:

y_{obs} = Observed response (area) for each concentration from each initial calibration standard

$\bar{y}$ = Mean observed response from the initial calibration

Y_i = Calculated (or predicted) response at each concentration from the initial calibration(s)

n = Total number of calibration points (i.e., 6 for a quadratic model; 7 for a third order model)

p = Number of adjustable parameters in the polynomial equation (i.e., 3 for a third order; 2 for a second order polynomial)

Under ideal conditions, with a "perfect" fit of the model to the data, the coefficient of the determination will equal 1.0. In order to be an acceptable non-linear calibration, the COD must be greater than or equal to 0.99.

As noted in Sec. 7.5, whichever of these options is employed, a unique analyte or surrogate concentration must fall within the calibration range. Analysts are advised to check both second and third order calibration models to ensure that this holds true (e.g., no parabolas or repeating functions in the calibration range). Samples with concentrations that exceed the calibration range must be diluted to fall within the range.

7.5.4 Data transformations

An understanding of the fundamental behavior of the detector may be used to choose a data transformation that will then allow for a simple calibration model. For example the response of a flame photometric detector in the sulfur mode is known to be proportional to the square of the sulfur concentration. Therefore, using the data system to take the square root of the instrument response before integration or the square root of the peak height allows for a calibration factor approach rather than a polynomial calibration curve. Instrument response may be transformed prior to any calculations (including integration) subject to the following constraints:

7.5.4.1 Any parameters used in the transformation should be fixed for the calibration and all subsequent analyses and verifications until the next calibration.

7.5.4.2 The transformation model chosen should be consistent with the behavior of the instrument and detector. All data transformations must be clearly defined and documented by the analyst and related back to the fundamental behavior of the detector. In other words, this approach may not be used in the absence of specific knowledge about the behavior of the detector, nor as a "shot in the dark" to describe the calibration.

7.5.4.3 No transformations should be performed on areas or other results (e.g., the transformation must be applied to the instrument response itself).

7.5.4.4 When the transformed data are used to develop calibration factors, those factors must meet the acceptance criteria described in Sec. 7.5.1.

7.6 Retention time windows

Retention time windows are crucial to the identification of target compounds. Absolute retention times are used for compound identification in all GC and HPLC methods that do *not* employ internal standard calibration. Retention time windows are established to compensate for minor shifts in absolute retention times as a result of sample loadings and normal chromatographic variability. The width of the retention time window should be carefully established to minimize the occurrence of both false positive and false negative results. Tight retention time windows may result in false negatives and/or may cause unnecessary reanalysis of samples when surrogates or spiked compounds are erroneously not identified. Overly wide retention time windows may result in false positive results that cannot be confirmed upon further analysis.

The following subsections describe <u>one</u> approach that may be used to establish retention time windows for GC and HPLC methods. Other approaches may be employed, provided that the analyst can demonstrate that they provide performance appropriate for the intended application.

<u>NOTE</u>: The criteria listed in Sec. 7.6 are provided for GC and HPLC procedures using non-MS or FTIR detection. Identification procedures are different for GC/MS (e.g., Methods 8260 and 8270), HPLC/MS (e.g., Methods 8321 and 8325), and GC/FT-IR (e.g., Method 8410).

7.6.1 Before establishing retention time windows, make sure that the chromatographic system is operating reliably and that the system conditions have been optimized for the target analytes and surrogates in the sample matrix to be analyzed. Make three injections of all single component standard mixtures and multi-component analytes (such as PCBs) over the course of a 72-hour period. Serial injections or injections over a period of less than 72 hours may result in retention time windows that are too tight.

7.6.2 Record the retention time for each single component analyte and surrogate to three decimal places (e.g., 0.007). Calculate the mean and standard deviation of the three absolute retention times for each single component analyte and surrogate. For multi-component analytes, choose three to five major peaks (see the determinative methods for more details) and calculate the mean and standard deviation of those peaks.

7.6.3 If the standard deviation of the retention times for a target compound is 0.000 (i.e., no difference between the absolute retention times), then the laboratory may either collect data from additional injections of standards or use a default standard deviation of 0.01 minutes. (Recording retention times to three decimal places rather than only two should minimize the instances in which the standard deviation is calculated as 0.000).

7.6.4 The width of the retention time window for each analyte, surrogate, and major constituent in multi-component analytes is defined as ± 3 times the standard deviation of the mean absolute retention time established during the 72-hour period. If the default standard deviation in Sec. 7.6.3 is employed, the width of the window will be 0.03 minutes.

7.6.5 Establish the center of the retention time window for each analyte and surrogate by using the absolute retention time for each analyte and surrogate from the calibration

verification standard at the beginning of the analytical shift. For samples run during the same shift as an initial calibration, use the retention time of the mid-point standard of the initial calibration.

7.6.6 The laboratory must calculate absolute retention time windows for each analyte and surrogate on each chromatographic column and instrument. New retention time windows must be established when a new GC column is installed. The retention time windows should be reported with the analysis results in support of the identifications made.

7.6.7 If the instrument data system is not capable of employing compound-specific retention time windows, then the analyst may choose the widest window and apply it to all compounds. As noted above, other approaches may also be employed, but must be documented by the analyst.

7.6.8 The surrogates are added to each sample, blank, and QC sample and are also contained in each calibration standard. Although the surrogates may be diluted out of certain sample extracts, their retention times in the calibration standards may be useful in tracking retention time shifts. Whenever the observed retention time of a surrogate is outside of the established retention time window, the analyst is advised to determine the cause and correct the problem before continuing analyses.

7.7 Calibration verification

The calibration relationship established during the initial calibration (Sec. 7.5) must be verified at periodic intervals. The process of calibration verification applies to both external standard and internal standard calibration techniques, as well as to linear and non-linear calibration models.

As a general rule, the initial calibration in an SW-846 method must be verified at the beginning of each 12-hour analytical shift during which samples are analyzed. (Some methods may specify more frequent verifications). The 12-hour analytical shift begins with the injection of the calibration verification standard (or the MS tuning standard in MS methods). The shift ends after the <u>completion</u> of the analysis of the last sample or standard that can be <u>injected</u> within 12 hours of the beginning of the shift.

If the response (or calculated concentration) for an analyte is within ±15% of the response obtained during the initial calibration, then the initial calibration is considered still valid, and the analyst may continue to use the CF or RF values from the initial calibration to quantitate sample results. The ±15% criterion may be superseded in certain determinative methods.

Except where the determinative method contains alternative calibration verification criteria, if the response (or calculated concentration) for any analyte varies from the mean response obtained during the initial calibration by more than ±15%, then the initial calibration relationship may no longer be valid.

<u>NOTE</u>: The process of calibration verification is fundamentally different from the approach called "continuing calibration" in some methods from other sources. As described in those methods, the calibration factors or response factors calculated during continuing calibration are used to update the calibration factors or response factors used for sample quantitation. This approach, while employed in other EPA programs, amounts to a daily single-point calibration, and is not appropriate nor permitted in SW-846 chromatographic procedures for trace environmental analyses.

In keeping with the approach described for initial calibration in Sec 7.5, if the average of the responses for all analytes is within 15%, then the calibration has been verified. However, the conditions in Sec. 7.5.1.2 also apply, e.g., the average must include all analytes in the calibration, regardless of whether they are target analytes for a specific project, and the data user must be provided with the calibration verification data or a list of those analytes that exceeded the 15% limit. The effect of using the average of the response for all analytes for calibration verification will be similar to that for the initial calibration – namely, that the quantitative results for those analytes where the difference is greater than 15% will include a greater uncertainty. The analyst and the data user should review the note in Sec. 7.5.1.2.

If the calibration does not meet the 15% limit (either on the basis of each compound or the average across all compounds), check the instrument operating conditions, and if necessary, restore them to the original settings, and inject another aliquot of the calibration verification standard. If the response for the analyte is still not within ±15%, then a new initial calibration must be prepared.

7.7.1 Verification of linear calibrations

Calibration verification for linear calibrations involves the calculation of the percent drift or the percent difference of the instrument response between the initial calibration and each subsequent analysis of the verification standard. Use the equations below to calculate % Drift or % Difference, depending on the procedure specified in the determinative method.

$$\% \text{ Drift} = \frac{\text{Calculated concentration} - \text{Theoretical concentration}}{\text{Theoretical concentration}} \times 100$$

where the calculated concentration is determined using the mean calibration factor or response factor from the initial calibration and the theoretical concentration is the concentration at which the standard was prepared.

$$\% \text{ Difference} = \frac{CF_v - \overline{CF}}{\overline{CF}} \times 100 \qquad \text{or} \qquad = \frac{RF_v - \overline{RF}}{\overline{RF}} \times 100$$

where CF_v and RF_v are the calibration factor and the response factor (whichever applies) from the analysis of the verification standard, and $\overline{CF}$ and $\overline{RF}$ are the mean calibration factor and mean response factor from the initial calibration. Except where superseded in certain determinative methods, the % difference or % drift calculated for the calibration verification standard must be within ±15% for each analyte, or averaged across all analytes (see Sec 7.7), before any sample analyses may take place.

7.7.2 Verification of a non-linear calibration

Calibration verification of a non-linear calibration is performed using the percent drift calculation described in Sec. 7.7.1, above. Except where superseded in certain determinative methods, the % drift calculated for the calibration verification standard must be within ±15% for each analyte, or averaged across all analytes (see Sec 7.7), before any sample analyses may take place. It may also be appropriate to employ two standards at different concentrations to verify the calibration. In this instance, one standard should be near the inflection point in the curve. The choice of specific standards and concentrations is generally a method- or project-specific consideration.

7.7.3 Regardless of whether a linear or non-linear calibration model is used, if either the percent drift or percent difference criterion is not met, then no sample analyses may take place until the calibration has been verified or a new initial calibration is performed that meets the specifications in Sec. 7.5 and those in the determinative method. If the calibration cannot be verified after the analysis of a single verification standard, then adjust the instrument operating conditions and/or perform instrument maintenance (see Sec. 7.11), and analyze another aliquot of the verification standard. If the calibration cannot be verified with the second standard, then a new initial calibration must be performed.

7.7.4 All target analytes and surrogates, including those reported as non-detects, must be included in a periodic calibration for purposes of retention time confirmation and to demonstrate that calibration verification criteria are being met. The frequency of this periodic calibration is project-, method-, and analyte-specific.

7.7.5 Calibration verification may be performed using both high and low concentration standards from time to time. This is particularly true when the ECD or ELCD is used. These detectors drift and are not as stable as FID or FPD, and periodic use of the high and low concentration standards serves as a further check on the initial calibration. The concentrations of these standards should generally reflect those observed in samples.

7.7.6 Additional analyses of the mid-point calibration verification standard during a 12-hour analytical shift are strongly recommended for methods involving external standard calibration. If the response for any analyte varies from the average initial calibration response by more than 15% in these additional determinations, corrective action (see Sec. 7.11) may be necessary to restore the system or a new calibration curve should be prepared for that compound.

The frequency of verification necessary to ensure accurate measurement is dependent on the detector and the sample matrix. Very sensitive detectors that operate in the sub-nanogram range are generally more susceptible to changes in response caused by column contamination and sample carryover. Therefore, more frequent verification of calibration (i.e., after every 10 samples) may be necessary for the electron capture, electrochemical conductivity, photoionization, and fluorescence detectors.

Sec. 8.2.2 specifies that samples analyzed using external standards must be bracketed by periodic analyses of standards that meet the QC acceptance criteria (e.g., calibration and retention time). Therefore, more frequent analyses of standards will minimize the number of sample extracts that would have to be reinjected if the QC limits are violated for the standard analysis. The results from these bracketing standards must meet the calibration verification criteria in Sec. 7.7.1 and 7.7.2 and the retention time criteria in Sec. 7.6. However, if the standard analyzed <u>after</u> a group of samples exhibits a response for an analyte that is <u>above</u> the acceptance limit, i.e., >15%, and the analyte was <u>not</u> detected in any of the previous samples during the analytical shift, then the sample extracts do not need to be reanalyzed, as the verification standard has demonstrated that the analyte would have been detected were it present.

7.7.7 Any method blanks specified in the preparative methods (Methods 3500 and 3600) may be run immediately after the calibration verification analyses to confirm that laboratory contamination does not cause false positive results, or at any other time during the analytical shift. If the method blank indicates contamination, then it may be appropriate to analyze a solvent blank to demonstrate that the contamination is not a result of carryover from standards or samples.

7.8 Chromatographic analysis of samples

7.8.1 Introduction of sample extracts into the chromatograph varies, depending on the volatility of the compound. Volatile organics are primarily introduced by purge-and-trap techniques (Method 5030, water and Method 5035, soils). However, the use of Method 5021, or another headspace technique, may be advisable for screening volatiles in some sample matrices to prevent overloading and contamination of the purge-and-trap system. Semivolatile and non-volatile analytes are introduced by direct or split/splitless injection.

7.8.1.1 Manual injection (GC)

Inject 1-5 µL of the sample extract. The use of the solvent flush technique is necessary for packed columns. Use 1-2 µL of sample extract for capillary columns.

7.8.1.2 Automated injection (GC)

Using automated injection, smaller volumes (i.e., 1 µL) may be injected, and the solvent flush technique is not necessary. Laboratories should demonstrate that the injection volume is reproducible.

7.8.1.3 Purge-and-trap

Refer to Methods 5000, 5030, or 5035 for details.

7.8.1.4 Manual injection (HPLC)

Inject 10-100 µL. This is generally accomplished by over-filling the injection loop of a zero-dead-volume injector. Larger volumes may be injected if better sensitivity is required, however, chromatographic performance may be affected.

7.8.1.5 Automated injection (HPLC)

Inject 10-100 µL. Laboratories should demonstrate that the injection volume is reproducible. Larger volumes may be injected if greater sensitivity is required, however, chromatographic performance may be adversely affected.

7.8.2 All analyses, including field samples, matrix spike samples, matrix spike duplicates, laboratory control samples, method blanks, and other QC samples, are performed during an analysis sequence. The sequence begins with instrument calibration, which is followed by the analysis of sample extracts. Verification of calibration and retention times is necessary no less than once every 12-hour analytical shift. The sequence ends when the set of samples has been injected or when qualitative and/or quantitative QC criteria are exceeded. As noted in Secs. 7.7.6 and 8.2.2, when employing external standard calibration, it is necessary that a calibration verification standard be run at the end of the sequence to bracket the sample analyses. Acceptance criteria for the initial calibration and calibration verification are described in Secs. 7.5 - 7.7.

Analysis of calibration verification standards every 10 samples is strongly recommended, especially for the highly sensitive GC and HPLC detectors that detect sub-nanogram concentrations. Frequent analysis of calibration verification standards helps ensure that chromatographic systems are performing acceptably and that false positives, false negatives and poor quantitations are minimized. Samples analyzed using external standard calibration

must be bracketed by the analyses of calibration standards that meet the QC limits for verification of calibration and retention times. If criteria are exceeded, corrective action must be taken (see Sec. 7.11) to restore the system and/or a new calibration curve must be prepared for that compound and the samples must be reanalyzed.

Certain methods may also include QC checks on column resolution, analyte degradation, mass calibration, etc., at the beginning of a 12-hour analytical shift.

7.8.3 Sample concentrations are calculated by comparing sample responses with the initial calibration of the system (Sec. 7.5). If sample response exceeds the limits of the initial calibration range, dilute the extract (or sample) and reanalyze. Extracts should be diluted so that all peaks are on scale, as overlapping peaks are not always evident when peaks are off scale. Computer reproduction of chromatograms, manipulated to ensure all peaks are on scale over a 100-fold range, is acceptable, as long as calibration limits are not exceeded. When overlapping peaks cause errors in peak area integration, the use of peak height measurements is recommended.

7.8.4 If chromatographic peaks are masked by the presence of interferences, further sample cleanup is necessary. See Method 3600 for guidance.

7.8.5 When there are a large number of target analytes, it may be difficult to fully resolve these compounds. Examples of chromatograms for the compounds of interest are provided in many determinative methods.

7.9 Compound Identification

Tentative identification of an analyte occurs when a peak from a sample extract falls within the daily retention time window. Confirmation is necessary when the composition of samples is not well characterized. Confirmation techniques include analysis on a second column with dissimilar stationary phase, by GC/MS (full scan or SIM) or HPLC/MS (if concentration permits), HPLC/UV data at two different wavelengths, GC or HPLC data from two different detectors, or by other recognized confirmation techniques. For HPLC/UV methods, the ability to generate UV spectra with a diode array detector may provide confirmation data from a single analysis, provided that the laboratory can demonstrate this ability for typical sample extracts (not standards) by comparison to another recognized confirmation technique.

When confirmation is made on a second column, that analysis should meet all of the QC criteria described above for calibration, retention times, etc. Confirmation is not required for GC/MS and HPLC/MS methods.

Confirmation may not be necessary if the composition of the sample matrix is well established by prior analyses, for instance, when a pesticide known to be produced or used in a facility is found in a sample from that facility.

When using GC/MS for confirmation, ensure that GC/MS analysis is performed on an extract at the appropriate pH for the analyte(s) being confirmed, i.e., do not look for basic analytes in an acidic extract. Certain analytes, especially pesticides, may degrade if extraction conditions were either strongly acidic and/or strongly basic.

Many chromatographic interferences result from co-elution of one or more compounds with the analyte of interest, or may be the result of the presence of a non-analyte peak in the retention time window of an analyte. Such co-elution problems affect quantitation as well as identification, and may

result in poor agreement between the quantitative results from two dissimilar columns. Therefore, even when the identification has been confirmed on a dissimilar column, the analyst should evaluate the agreement of the quantitative results on both columns, as described in Sec. 7.10.4.

7.10 Calculations

The calculation of sample results depends on the type of calibration (external or internal standard) and the calibration model employed (linear or non-linear). The following sections describe the calculations used in each instance. Specific determinative methods may contain additional information.

7.10.1 External standard calibration - linear calibration

The concentration of each analyte in the sample is determined by comparing the detector response (peak area or height) to the response for that analyte in the initial calibration. The concentration of an analyte may be calculated as follows, depending on the sample matrix:

7.10.1.1 Aqueous samples

$$\text{Concentration } (\mu g/L) = \frac{(A_s)(V_t)(D)}{(\overline{CF})(V_i)(V_s)}$$

where:

A_s = Area (or height) of the peak for the analyte in the sample.

V_t = Total volume of the concentrated extract (µL). For purge-and-trap analysis, V_t is not applicable and therefore is set at 1.

D = Dilution factor, if the sample or extract was diluted prior to analysis. If no dilution was made, D = 1. The dilution factor is always dimensionless.

$\overline{CF}$ = Mean calibration factor from the initial calibration (area per ng).

V_i = Volume of the extract injected (µL). The nominal injection volume for samples and calibration standards must be the same. For purge-and-trap analysis, V_i is not applicable and therefore is set at 1. If concentration units are used in calculating the calibration factor (see Sec. 7.4.2.1), then V_i is not used in this equation.

V_s = Volume of the aqueous sample extracted or purged in mL. If units of liters are used for this term, multiply the results by 1000.

Using the units specified here for these terms will result in a concentration in units of ng/mL, which is equivalent to µg/L.

7.10.1.2 Nonaqueous samples

$$\text{Concentration } (\mu g/kg) = \frac{(A_s)(V_t)(D)}{(\overline{CF})(V_i)(W_s)}$$

where A_s, V_t, D, $\overline{CF}$, and V_i are as described in 7.10.1.1, and

W_s = Weight of sample extracted or purged (g). Either the wet weight or dry weight may be used, depending upon the specific application of the data. If units of kilograms are used for this term, multiply the results by 1000.

Using the units specified here for these terms will result in a concentration in units of ng/g, which is equivalent to µg/kg.

For purge-and-trap analyses where a volume of methanol extract is added to organic-free reagent water and purged, V_t is the total volume of the methanol extract and V_i is the volume of methanol extract that is added to the 5 mL of organic-free reagent water.

7.10.1.3 If a linear calibration that does not pass through the origin has been employed, then the regression equation is rearranged as shown in Sec. 7.5.2, and the concentration of the analyte is calculated from the area response (y), the slope (a), and the intercept (b). When using this form of linear calibration, it is the laboratory's responsibility to ensure that the calculations take into account the volume or weight of the original sample, the dilution factor (if any), and dry weight (as applicable). One approach to this calculation is to perform the original linear regression using the concentration of the analyte in the final extract volume or the volume purged. The concentration of the analyte in the sample may then be calculated as follows:

$$C_s = \frac{(C_{ex})(V_t)}{(V_s)}$$

where:

C_s = Concentration in the sample
C_{ex} = Concentration in the final extract
V_t = Total volume of the concentrated extract
V_s = Volume of the sample extracted or purged

For solid samples, substitute the weight of the sample, W_s, for V_s.

For purge-and-trap analyses, the concentration of the analyte in the volume of the sample that is purged will be the same as in the original sample, except when dilutions are performed.

7.10.2 Internal standard calibration - linear calibration

The concentration of each analyte in the sample is calculated using the results of the initial calibration, according to one of the following sections, depending on the sample matrix:

7.10.2.1 Aqueous samples

$$\text{Concentration } (\mu g/L) = \frac{(A_s)(C_{is})(D)(V_i)}{(A_{is})(\overline{RF})(V_s)(1000)}$$

where:

A_s = Area (or height) of the peak for the analyte in the sample.

A_{is} = Area (or height) of the peak for the internal standard.

C_{is} = Concentration of the internal standard in the concentrated sample extract or volume purged in µg/L.

D = Dilution factor, if the sample or extract was diluted prior to analysis. If no dilution was made, D = 1. The dilution factor is always dimensionless.

V_i = Volume of the extract injected (µL). The nominal injection volume for samples and calibration standards must be the same. For purge-and-trap analysis, V_i is not applicable and is set at 1.

$\overline{RF}$ = Mean response factor from the initial calibration. Unlike calibration factors for external standard calibration, the response factor is dimensionless (see Sec. 7.5).

V_s = Volume of the aqueous sample extracted or purged (mL). If units of liters are used for this term, multiply the results by 1000.

The 1000 in the denominator represents the number of µL in 1 mL. If the injection (V_i) is expressed in mL, then the 1000 may be omitted.

Using the units specified here for these terms will result in a concentration in units of ng/mL, which is equivalent to µg/L.

7.10.2.2 Nonaqueous samples

$$\text{Concentration } (\mu g/kg) = \frac{(A_s)(C_{is})(D)(V_i)}{(A_{is})(\overline{RF})(W_s)(1000)}$$

where: A_s, A_{is}, C_{is}, D, and $\overline{RF}$ are the same as for aqueous samples, and

W_s = Weight of sample extracted (g). Either a dry weight or wet weight may be used, depending upon the specific application of the data. If units of kilograms are used for this term, multiply the results by 1000.

The 1000 in the denominator represents the number of µL in 1 mL. If the injection (V_i) is expressed in mL, then the 1000 may be omitted.

Using the units specified here for these terms will result in a concentration in units of ng/g, which is equivalent to µg/kg.

7.10.2.3 If a linear calibration that does not pass through the origin has been employed, then the regression equation is rearranged in a fashion similar to that described in Sec. 7.10.1.3.

7.10.3 Calculations for a non-linear calibration curve

When a non-linear curve has been employed, the non-linear model is rearranged to solve for the concentration of the analyte in the extract or purge volume, and the extract concentration is converted to a sample concentration in a fashion similar to that described in Sec. 7.10.1.3.

When non-linear calibrations are employed, it is essential that the laboratory clearly document the calculation of analyte concentrations. Example calculations should be reported that clearly indicate how the instrument response (area) was converted into a sample result.

7.10.4 Comparison between results from different columns or detectors

When sample results are confirmed using two dissimilar columns or with two dissimilar detectors, the agreement between the quantitative results should be evaluated after the identification has been confirmed. Calculate the relative percent difference (RPD) between the two results using the formula below.

$$RPD = \frac{|R_1 - R_2|}{\left(\frac{R_1 + R_2}{2}\right)} \times 100$$

where R_1 and R_2 are the results on the two columns and the vertical bars in the equation above indicate the absolute value of the difference. Therefore, the RPD is always a positive value.

7.10.4.1 If one result is significantly higher (e.g., >40%), check the chromatograms to see if an obviously overlapping peak is causing an erroneously high result. If no overlapping peaks are noted, examine the baseline parameters established by the instrument data system (or operator) during peak integration.

7.10.4.2 If no anomalies are noted, review the chromatographic conditions. If there is no evidence of chromatographic problems, report the higher result. This approach is conservative relative to protection of the environment. The data user should be advised of the disparity between the results on the two columns.

7.11 Suggested chromatographic system maintenance

Corrective measures may involve any one or more of the following remedial actions. This list is by no means comprehensive and analysts should develop expertise in troubleshooting their specific instruments and analytical procedures. The manufacturers of chromatographic instruments, detectors, columns, and accessories generally provide detailed information regarding the proper operation and limiting factors associated with their products. The importance of reading and reviewing this information cannot be over-emphasized.

7.11.1 Capillary GC columns

Routine maintenance may compel the analyst to clean and deactivate the glass injection port insert or replace it with a fresh insert that has been cleaned and deactivated with dichlorodimethylsilane. Cut off 0.5 - 1.0 m of the injector end of the column using a 90° cut. Place ferrule onto the column before cutting.

Exceptional maintenance may compel the analyst to replace gas traps and backflush the column with solvent according to the manufacturer's instructions. If these procedures fail to eliminate the degradation problem, it may be necessary to deactivate the metal injector body and/or replace the column.

7.11.2 Metal (GC) injector body

Turn off the oven and remove the analytical column when the oven has cooled. Remove the glass injection port insert. Lower the injection port temperature to room temperature. Inspect the injection port and remove any noticeable foreign material.

Place a beaker beneath the injector port inside the GC oven. Using a wash bottle, serially rinse the entire inside of the injector port with acetone and then toluene, catching the rinsate in the beaker.

Prepare a solution of deactivating agent (dichlorodimethylsilane) following manufacturer's directions. After all metal surfaces inside the injector body have been thoroughly coated with the deactivation solution, serially rinse the injector body with toluene, methanol, acetone, and hexane. Reassemble the injector and replace the GC column.

7.11.3 HPLC columns

Examine the system and check for drips that are indicative of plumbing leaks. Check that tubing connectors are of the shortest possible length to minimize dead volumes and reduce band broadening. Compatible guard columns should be installed to protect analytical columns.

If degradation of resolution or changes in back pressure are observed, the first action should be to replace the guard column if one is installed. Secondly, temporarily reverse the flow through the column to dislodge contamination in the frit with the column disconnected from the detector. If this does not correct the problem, place the analytical column in a vise, remove the inlet compression fitting and examine the column.

Analysts should establish that no void volume has developed, that the column packing has not become contaminated, and that the frit is not clogged. Void volumes can be filled with compatible packing and frits replaced.

Columns must eventually be replaced as the bonding and end-capping groups used to modify the silica are lost with time. Loss of these groups will result in chromatographic tailing and changes in analyte retention times. Retention times may also change because of differences in column temperature or because the composition of the solvent gradient is not completely reproducible.

8.0 QUALITY CONTROL

8.1 Refer to Chapter One for specific quality control procedures. The development of in-house QC limits for each method is encouraged, as described in Sec. 8.7. The use of instrument-specific QC limits is encouraged, provided such limits will generate data appropriate for use in the intended application. In general, the following QC requirements pertain to all the determinative methods listed in Sec. 1.1 unless superseded by specific requirements provided in the determinative method.

8.2 Evaluating chromatographic performance

The analyst's expertise in performing chromatography is a critical element in the successful performance of chromatographic methods. Successful generation of data requires selection of suitable preparation and analysis methods and an experienced staff to use these methods.

8.2.1 For each 12-hour period during which analysis is performed, the performance of the entire analytical system should be checked. These checks should be part of a formal quality control program that includes the analysis of blanks, calibration standards, matrix spikes, laboratory control samples and replicate samples, although all of these checks need not be performed during each shift.

8.2.2 Ongoing data quality checks are compared with established performance criteria to determine if the results of analyses meet the performance characteristics of the method. Therefore, all sample analyses performed using external standard calibration must be bracketed with acceptable calibration verification standards.

8.2.3 In addition to the quantitative measures of comparison described below and in the individual methods, analysts should evaluate chromatograms and instrument operation. Questions that should be asked include:

Do the peaks look normal (Gaussian)?
Is the response obtained comparable to the response from previous calibrations?
Do the column fittings need tightening?
Are non-target peaks present in calibration analyses?
Are contaminants present in the blanks?
Is the injector leaking (e.g., does the GC injector septum need replacing)?
Does the HPLC guard column need replacement?

8.2.4 Significant peak tailing, leaks, changes in detector response and laboratory contamination should be corrected. Tailing problems are generally traceable to active sites on the column, cold spots in a GC, improper choice of HPLC mobile phase, the detector inlet, or leaks in the system.

8.2.5 Recalibration of the instrument must take place when the performance changes to the point that the calibration verification acceptance criteria (Sec. 7.7) cannot be achieved. In addition, significant maintenance activities or hardware changes may also require recalibration. The sections below provide general guidance on the sorts of procedures that may or may not require recalibration.

8.2.5.1 There are various types of instrument maintenance that should <u>not</u> automatically require recalibration of the instrument. Examples include changing: septa; compressed gas cylinders; syringes; moisture, hydrocarbon, or oxygen traps; solvents in an ELCD; purge tubes; PTFE transfer lines; glow plugs; split seals; column fittings; inlets; or filaments. Other procedures include breaking off or changing a guard column or cleaning the inlet. Whenever such procedures are performed, the analyst must demonstrate that the results for a calibration verification standard meet the acceptance criteria in Sec. 7.7. before the analysis of any samples. Otherwise, recalibration is required.

8.2.5.2 In contrast to Sec. 8.2.5.1, some maintenance procedures are so likely to affect the instrument response that recalibration is <u>automatically</u> required, regardless

of the ability to meet the calibration verification acceptance criteria. These procedures include: changing, replacing, or reversing the column; recoating the bead in a detector; changing nitrogen tubes in an NPD; changing resins; changing the PID seal or lamp; changing the FID jet; changing the entrance lens, draw out lens, or repeller; cleaning the MS source; changing the electron multiplier, ion source chamber, or injector port. Whenever such procedures are performed, the analyst must perform a new initial calibration that meets the requirement using Sec 7.5. As noted in Sec. 7.6, changing or replacing the column will also require that the retention time windows be redetermined.

8.2.6 The analysis of method blanks is critical to the provision of meaningful sample results. Consult the appropriate 3500 or 5000 series method for the specifics of the preparation of method blanks. The following general guidelines apply to the interpretation of method blank results.

8.2.6.1 Method blanks should be prepared at a frequency of at least 5%, that is, one method blank for each group of up to 20 samples prepared at the same time, by the same procedures. For samples analyzed for volatiles by the purge-and-trap technique, the preparation is equivalent to the analysis. Therefore, one purge-and-trap method blank must be analyzed with each group of up to 20 samples analyzed on the same instrument during the same analytical shift.

8.2.6.2. When samples that are extracted together are analyzed on separate instruments or on separate analytical shifts, the method blank associated with those samples (e.g., extracted with the samples) must be analyzed on at least one of those instruments. A solvent blank should be analyzed on all other instruments on which the set of samples were analyzed to demonstrate that the instrument is not contributing contaminants to the samples.

8.2.6.3 Unless otherwise described in a determinative method, the method blank may be analyzed immediately after the calibration verification standard, to ensure that there is no carryover from the standard, or at another point in the analytical shift.

8.2.6.4 When sample extracts are subjected to cleanup procedures, the associated method blank must also be subjected to the same cleanup procedures.

8.2.6.5 As described in Chapter One, the results of the method blank should be:

8.2.6.5.1 Less than the laboratory's MDL for the analyte or less than the level of acceptable blank contamination specified in the approved quality assurance project plan.

8.2.6.5.2 Less than 5% of the regulatory limit associated with an analyte.

8.2.6.5.3 Or less than 5% of the sample result for the same analyte, whichever is greater.

8.2.6.5.4 If the method blank results do not meet the acceptance criteria above, then the laboratory should take corrective action to locate and reduce the source of the contamination and to re-extract and reanalyze any samples associated with the contaminated method blank.

8.2.6.6 The laboratory should not subtract the results of the method blank from those of any associated samples. Such "blank subtraction" is inappropriate for the GC and HPLC methods addressed here, and often leads to negative sample results. If the method blank results do not meet the acceptance criteria in 8.2.6.5 and reanalysis is not practical, then the data user should be provided with the sample results, the method blank results, and a discussion of the corrective actions undertaken by the laboratory.

8.2.6.7 Method blanks and/or solvent blanks may also be used to check for contamination by carryover from a high-concentration sample into subsequent samples (see Sec. 3.1). When the analysis of such blanks is not possible, such as when an unattended autosampler is employed, the analyst should review the results for at least the next two samples after the high-concentration sample. If analytes in the high-concentration sample are not present in the subsequent samples, then the lack of carryover has been demonstrated. If there is evidence that carryover may have occurred, then the samples should be reanalyzed.

8.3 Summary of required instrument QC

The following criteria primarily pertain to GC and HPLC methods with non-MS or FTIR detectors, and may be superseded by criteria specified in individual determinative methods (e.g., Methods 8021, 8260, 8270, 8321, 8325, and 8410).

8.3.1 The criteria for linearity of the initial calibration curve is an RSD of $\leq$ 20%.

8.3.2 For non-linear calibration curves, the coefficient of the determination (COD) must be greater than or equal to 0.99 (see Sec. 7.5.2).

8.3.3 Retention time (RT) windows must be established for the identification of target analytes. See Sec. 7.6 for guidance on establishing the absolute RT windows.

8.3.4 The retention times of all analytes in all verification standards must fall within the absolute RT windows. If an analyte falls outside the RT window in a calibration verification standard, new absolute RT windows must be calculated, unless instrument maintenance corrects the problem.

8.3.5 The calibration verification results must be within $\pm$ 15% of the response calculated using the initial calibration. If the limit is exceeded, a new standard curve must be prepared unless instrument maintenance corrects the problem.

8.4 Initial demonstration of proficiency

Each laboratory must demonstrate initial proficiency with each combination of sample preparation and determinative methods that it utilizes, by generating data of acceptable accuracy and precision for a reference sample containing the target analytes in a clean matrix. The laboratory must also repeat this demonstration whenever new staff are trained or significant changes in instrumentation are made.

8.4.1 The reference samples are prepared from a spiking solution containing each analyte of interest. The reference sample concentrate (spiking solution) may be prepared from pure standard materials, or purchased as certified solutions. If prepared by the laboratory, the reference sample concentrate must be made using stock standards prepared independently from those used for calibration.

Preparation of the reference sample concentrate is dependent upon the method being evaluated. Guidance for reference sample concentrations for certain methods are listed in Sec. 8.0 of Methods 3500 and 5000. In other cases, the determinative methods contain guidance on preparing the reference sample concentrate and the reference sample. If no guidance is provided, prepare a reference sample concentrate in methanol (or any water miscible solvent) at a concentration such that the spike will provide a concentration in the clean matrix that is 10 - 50 times the MDL for each analyte in that matrix.

The concentration of target analytes in the reference sample may be adjusted to more accurately reflect the concentrations that will be analyzed by the laboratory. If the concentration of an analyte is being evaluated relative to a regulatory limit or action level, see Sec. 8.5.1 for information on selecting an appropriate spiking level.

8.4.2 To evaluate the performance of the total analytical process, the reference samples must be handled in exactly the same manner as actual samples. Use a clean matrix for spiking purposes (one that does not have any target or interference compounds), e.g., organic-free reagent water for the aqueous matrix and organic-free sand or soil for the solid matrix.

8.4.3 Preparation of reference samples

8.4.3.1 Volatile organic analytes

Prepare the reference sample by adding 200 µL of the reference sample concentrate (Sec. 8.4.1) to 100 mL of organic-free reagent water. Transfer this solution immediately to a 20- or 25-mL (or four 5-mL) gas-tight syringe(s) when validating water analysis performance by Method 5030. Alternatively, the reference sample concentrate may be injected directly through the barrel of the 5- or 25-mL syringe. See Method 5000 (Sec. 8.0) for guidance on other preparative methods and matrices.

8.4.3.2 Semivolatile and nonvolatile organic analytes

Prepare the reference sample by adding 1.0 mL of the reference sample concentrate (Sec. 8.4.1) to each of four 1-L aliquots of organic-free reagent water. See Method 3500 (Sec. 8.0) for other matrices.

8.4.4 Analyze at least four replicate aliquots of the well-mixed reference samples by the same procedures used to analyze actual samples (Sec. 7.0 of each of the methods). This will include a combination of the sample preparation method (usually a 3500 series method for extractable organics or a 5000 series method for volatile organics) and the determinative method (an 8000 series method).

8.4.5 Calculate the average recovery ($\bar{x}$) in µg/L, and the standard deviation of the recovery (s) in µg/L, for each analyte of interest using the four results.

8.4.6 Multiple-laboratory performance data are included in some determinative methods and may be used as guidance in evaluating performance in a single laboratory. Compare s and $\bar{x}$ for each analyte with the corresponding performance data for precision and accuracy given in the performance data table at the end of the determinative method. If s and $\bar{x}$ for all analytes of interest meet the appropriate acceptance criteria, then the system performance is acceptable and analysis of actual samples can begin. If any individual s value exceeds the

precision limit or any individual $\bar{x}$ value falls outside the range for accuracy, then the system performance may be unacceptable for that analyte.

NOTE: The large number of analytes in each of the methods presents a substantial probability that one or more analyte will fail at least one of the performance criteria when all analytes of a given method are determined.

When one or more of the analytes fail at least one of the performance criteria, the analyst should proceed according to Sec. 8.4.6.1 or 8.4.6.2.

8.4.6.1 Locate and correct the source of the problem and repeat the test for all analytes of interest, beginning at Sec. 8.4.2.

8.4.6.2 Beginning at Sec. 8.4.2, repeat the test only for those analytes that failed to meet criteria. Repeated failure, however, will confirm a general problem with the measurement system. If this occurs, locate and correct the source of the problem and repeat the test for all compounds of interest beginning at Sec. 8.4.2.

8.4.7 The performance data in many of the methods are based on single-laboratory performance. As with the multiple-laboratory data, the criteria in those methods may be used as guidance when evaluating laboratory performance. When comparing your laboratory data to performance data developed from single-laboratory data, certain analytes may be outside the limits, however, the majority should be within the acceptance limits.

8.4.8 Even when the determinative method contains performance data (either multiple-laboratory or single-laboratory), the development of in-house acceptance limits is strongly recommended, and may be accomplished using the general considerations described in Sec. 8.7.

8.4.9 In the absence of recommended acceptance criteria for the initial demonstration of proficiency, the laboratory should use recoveries of 70 - 130% as guidance in evaluating the results. Given that the initial demonstration is performed in a clean matrix, the average recoveries of analyte from the four replicates should generally fall within this range. In addition, since the laboratory will repeat the initial demonstration of proficiency whenever new staff are trained or significant changes in instrumentation are made, the resulting data should be used to develop in-house acceptance criteria, as described in Sec. 8.7.

8.5 Matrix spike and laboratory control samples

The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision, accuracy, and detection limit). At a minimum, this will include the analysis of at least one matrix spike and one duplicate unspiked sample or one matrix spike/matrix spike duplicate (MS/MSD) pair with each batch of up to 20 samples of the same matrix processed together (see Chapter One). If samples are expected to contain the target analytes of concern, then laboratories may use one matrix spike and a duplicate analysis of an unspiked field sample as an alternative to the MS/MSD pair (see Sec. 8.5.3).

In the case of purge-and-trap methods, the MS/MSD, or MS and duplicate samples, should be prepared and analyzed concurrently with the samples. In the case of samples that involve an extraction procedure, the MS/MSD, or MS and duplicate samples, should be extracted with the batch of samples but may be analyzed at any time.

In addition, a Laboratory Control Sample (LCS) should be included with each analytical batch. The LCS consists of an aliquot of a clean (control) matrix similar to the sample matrix and of the same weight or volume. The LCS is spiked with the same analytes at the same concentrations as the matrix spike. When the results of the matrix spike analysis indicates a potential problem due to the sample matrix itself, the LCS results are used to verify that the laboratory can perform the analysis in a clean matrix.

The concentration of the matrix spike sample and/or the LCS should be determined as described in Secs. 8.5.1 and 8.5.2.

8.5.1 If, as in compliance monitoring, the concentration of a specific analyte in the sample is being checked against a regulatory concentration limit or action level, the spike should be at or below the limit, or 1 - 5 times the background concentration (if historical data are available), whichever concentration is higher.

If historical data are not available, it is suggested that a background sample of the same matrix from the site be submitted for matrix spiking purposes to ensure that high concentrations of target analytes and/or interferences will not prevent calculation of recoveries.

8.5.2 If the concentration of a specific analyte in a sample is not being checked against a limit specific to that analyte, then the analyst may spike the sample at the same concentration as the reference sample (Sec. 8.4.1), at 20 times the estimated quantitation limit (EQL) in the matrix of interest, or at a concentration near the middle of the calibration range. It is again suggested that a background sample of the same matrix from the site be submitted as a sample for matrix spiking purposes.

8.5.3 To develop precision and accuracy data for each of the spiked compounds, the analyst has two choices: analyze the original sample, and an MS/MSD pair; or analyze the original sample, a duplicate sample, and one spiked sample. If samples are not expected to contain the target analytes of concern, then the laboratory may use a matrix spike and matrix spike duplicate pair. If samples are expected to contain the target analytes of concern, then the laboratory may use one matrix spike and a duplicate analysis of an unspiked field sample as an alternative to the MS/MSD pair.

Begin by analyzing one sample aliquot to determine the background concentration of each analyte. Prepare a matrix spike concentrate according to one of the options specified in Sec. 8.5.1 or 8.5.2.

Prepare a matrix spike sample by adding the appropriate volume of the matrix spike concentrate to another aliquot of the sample to yield the desired concentration (see Secs. 8.5.1 and 8.5.2). Prepare a matrix spike duplicate sample from a third aliquot of the sample.

Analyze the MS/MSD samples using the same procedures employed for the original sample, and calculate the concentration of each analyte in the matrix spike and matrix spike duplicate. Likewise, analyze the LCS samples using the same procedures employed for the original sample, and calculate the concentration of each analyte in the LCS.

8.5.3.1 Calculation of recovery

Accuracy is estimated from the recovery of spiked analytes from the matrix of interest. Laboratory performance in a clean matrix is estimated from the recovery of

analytes in the LCS. Calculate the recovery of each spiked analyte in the matrix spike, matrix spike duplicate (if performed) and LCS according to the following formula.

$$\text{Recovery} = \%R = \frac{C_s - C_u}{C_n} \times 100$$

where:

C_s = Measured concentration of the spiked sample aliquot
C_u = Measured concentration of the unspiked sample aliquot (use 0 for the LCS)
C_n = Nominal (theoretical) concentration increase that results from spiking the sample, or the nominal concentration of the spiked aliquot (for LCS)

8.5.3.2 Calculation of precision

Precision is estimated from the relative percent difference (RPD) of the concentrations (*not* the recoveries) measured for matrix spike/matrix spike duplicate pairs, or for duplicate analyses of unspiked samples. Calculate the RPD according to the formula below.

$$RPD = \frac{|C_1 - C_2|}{\left(\frac{C_1 + C_2}{2}\right)} \times 100$$

where:

C_1 = Measured concentration of the first sample aliquot
C_2 = Measured concentration of the second sample aliquot

8.5.4 Recommended QC acceptance criteria for matrix spike samples and LCS

It is necessary for the laboratory to develop single-laboratory performance data for accuracy and precision in the matrices of interest (see Sec. 8.7). In addition, laboratories should monitor method performance in each matrix, through the use of control charts and other techniques.

Many methods may not contain recommended acceptance criteria for LCS results. The laboratory should use 70 - 130% as interim acceptance criteria for recoveries of spiked analytes, until in-house LCS limits are developed (see Sec. 8.7). Where in-house limits have been developed for matrix spike recoveries, the LCS results should fall within those limits, as the LCS is prepared in a clean matrix.

Even where the determinative methods provide performance criteria for matrix spikes and LCS, it is necessary for laboratories to develop in-house performance criteria and compare them to those in the methods. The development of in-house performance criteria is discussed in Sec. 8.7.

As a general rule, the recoveries of most compounds spiked into samples should fall within the range of 70 - 130%, and this range should be used as a guide in evaluating in-house performance. However, as described in Sec. 8.5.4.1, matrix spike recoveries and LCS recoveries may be affected by the spike-to-background ratio.

Where methods do contain performance data for the matrix of interest, use Secs. 8.5.4.1 and 8.5.4.2 as guidance in evaluating data generated by the laboratory.

8.5.4.1 When multiple-laboratory performance data for the matrix of interest are provided in the determinative method, compare the percent recovery (%R) for each analyte in a water sample with the performance data. Given that such performance criteria were developed from multi-laboratory data, they should be met in almost all laboratories. See Sec. 8.7.10 for more information on comparisons between limits. The performance data include an allowance for error in measurement of both the background and spike concentrations, and assume a spike-to-background ratio of 5:1. If spiking was performed at a concentration lower than that used for the reference sample (Sec. 8.4), the analyst may use either the performance data presented in the tables, or laboratory-generated QC acceptance criteria calculated for the specific spike concentration, provided that they meet the project-specific data quality objectives.

8.5.4.2 When the sample was spiked at a spike-to-background ratio other than 5:1, the laboratory should calculate acceptance criteria for the recovery of an analyte. Some determinative methods contain a table entitled "Method Accuracy and Precision as a Function of Concentration" which gives equations for calculating accuracy and precision as a function of the spiking concentration. These equations may be used as guidance in establishing the acceptance criteria for matrix spike samples.

The equations are the result of linear regression analyses of the performance data from a multiple-laboratory study. The equations are of the form:

$$\text{Accuracy} = x' = (a)C + b$$

where a is a number less than 1.0, b is a value greater than 0.0, and C is the test concentration (or true value).

Performance criteria for accuracy may be calculated from these equations by substituting the spiking concentration used by the laboratory in place of "C," and using the values of a and b given in the table for each analyte.

Performance criteria for precision are calculated in a similar fashion, using the a and b values for precision given in the table for each analyte. Precision may be calculated as single analyst precision, or overall precision, using the appropriate equations from the table. An acceptable performance range may be calculated for each analyte as:

$$\text{Acceptance range } (\mu g/L) = \text{Accuracy} \pm (2.44)\text{Precision}$$

8.5.5 Also compare the recovery data from the matrix spike with the LCS data (use the average recovery if a matrix spike and matrix spike duplicate were analyzed). If any individual percent recovery in the matrix spike (or matrix spike duplicate) falls outside the designated range for recovery, the laboratory should determine if there is a matrix effect or a laboratory performance problem. A matrix effect is indicated if the LCS data are within limits but the matrix spike data exceed the limits. The surrogate recovery data (Sec. 8.6) should also be used to evaluate the data. Recoveries of both matrix spike compounds and surrogates that are outside of the acceptance limits suggest more pervasive analytical problems then problems with the recoveries of either matrix spikes or surrogates alone.

8.6 Surrogate recoveries

8.6.1 It is necessary that the laboratory evaluate surrogate recovery data from individual samples versus surrogate recovery limits developed in the laboratory. The general considerations for developing in-house acceptance criteria for surrogate recoveries are described in Sec. 8.7.

8.6.2 Surrogate recovery is calculated as:

$$\text{Recovery (\%)} = \frac{\text{Concentration (or amount) found}}{\text{Concentration (or amount) added}} \times 100$$

If recovery is not within in-house surrogate recovery limits, the following procedures are necessary.

8.6.2.1 Check to be sure that there are no errors in the calculations, surrogate solutions or internal standards. If errors are found, recalculate the data accordingly. Examine chromatograms for interfering peaks and integrated peak areas.

8.6.2.2 Check instrument performance. If an instrument performance problem is identified, correct the problem and re-analyze the extract (or re-analyze the sample for volatiles).

8.6.2.3 Some samples may require dilution in order to bring one or more target analytes within the calibration range or to overcome significant interferences with some analytes. This may result in the dilution of the surrogate responses to the point that the recoveries can not be measured. If the surrogate recoveries are available from a less-diluted or undiluted aliquot of the sample or sample extract, those recoveries may be used to demonstrate that the surrogates were within the QC limits, and no further action is required. However, the results of both the diluted and undiluted (or less-diluted) analyses should be provided to the data user.

8.6.2.4 If no instrument problem is found, the sample should be re-extracted and re-analyzed (or re-analyze the sample for volatiles).

8.6.2.5 If, upon re-analysis (in either 8.6.2.2 or 8.6.2.4), the recovery is again not within limits, report the data as an "estimated concentration." If the recovery is within the limits in the re-analysis, provide the re-analysis data to the data user. If the holding time for the method has expired prior to the re-analysis, provide both the original and re-analysis results to the data user, and note the holding time problem.

8.7 Generating performance criteria for matrix spike recoveries, surrogate recoveries, initial demonstration of proficiency, and laboratory control sample recoveries

It is essential that laboratories calculate in-house performance criteria for matrix spike recoveries and surrogate recoveries. It may also be useful to calculate such in-house criteria for laboratory control sample (LCS) recoveries and for the initial demonstration of proficiency when experience indicates that the criteria recommended in specific methods are frequently missed for some analytes or matrices. The development of in-house performance criteria and the use of control charts or similar procedures to track laboratory performance cannot be over-emphasized. Many data systems and commercially-available software packages support the use of control charts.

The procedures for the calculation of in-house performance criteria for matrix spike recovery and surrogate recovery are provided below. These procedures may also be applied to the development of in-house criteria for the initial demonstration of proficiency and for LCS recoveries.

8.7.1 For each matrix spike sample analyzed, calculate the percent recovery of each matrix spike compound added to the sample, in a fashion similar to that described in Sec. 8.5.3.3. For each field sample, calculate the percent recovery of each surrogate as described in Sec. 8.6.

8.7.2 Calculate the average percent recovery (p) and the standard deviation (s) for each of the matrix spike compounds after analysis of 15-20 matrix spike samples of the same matrix, using the equations in Sec. 7.5.1, as guidance. Calculate the average percent recovery (p) and the standard deviation (s) for each of the surrogates after analysis of 15-20 field samples of the same matrix, in a similar fashion.

8.7.3 After the analysis of 15-20 matrix spike samples of a particular matrix (or matrix spike limits) or 15-20 field samples (for surrogate limits), calculate upper and lower control limit for each matrix spike or surrogate compound:

Upper control limit = p + 3s
Lower control limit = p - 3s

Calculate warning limits as:

Upper warning limit = p + 2s
Lower warning limit = p - 2s

For laboratories employing statistical software to determine these limits, the control limits approximate a 99% confidence interval around the mean recovery, while the warning limits approximate a 95% confidence interval.

8.7.4 Any matrix spike, surrogate, or LCS results outside of the control limits require evaluation by the laboratory. Such actions should begin with a comparison of the results from the samples or matrix spike samples with the LCS results. If the recoveries of the analytes in the LCS are outside of the control limits, then the problem may lie with the application of the extraction and/or cleanup procedures applied to the sample matrix or with the chromatographic procedures. Once the problem has been identified and addressed, corrective action may include the reanalysis of samples, or the extraction and analysis of new sample aliquots, including new matrix spike samples and LCS.

When the LCS results are within the control limits, the problem may either be related to the specific sample matrix or to an inappropriate choice of extraction, cleanup, and determinative methods. If the results are to be used for regulatory compliance monitoring, then the analyst must take steps to demonstrate that the analytes of concern can be determined in the sample matrix at the levels of interest.

The laboratory may use the warning limits to guide internal evaluations of method performance, track the performance of individual analysts, and monitor the effects of changes to the analytical procedures. Repeated results outside of the warning limits should lead to further evaluation.

8.7.5 Once established, control limits and warning limits for matrix spike compounds should be reviewed after every 10-20 matrix spike samples of the same matrix, and updated at least semi-annually. Control limits and warning limits for surrogates should be reviewed after every 20-30 field samples of the same matrix, and should be updated at least semi-annually. The laboratory should track trends in both performance and in the control limits themselves. The control and warning limits used to evaluate the sample results should be those in place at the time that the sample was analyzed. Once limits are updated, those limits should apply to all subsequent analyses of new samples.

8.7.6 For methods and matrices with very limited data (e.g., unusual matrices not analyzed often), interim limits should be established using available data or by analogy to similar methods or matrices.

8.7.7 Results used to develop acceptance criteria should meet all other QC criteria associated with the determinative method. For instance, matrix spike recoveries from a GC/MS procedure should be generated from samples analyzed after a valid GC/MS tune and a valid initial calibration that includes the matrix spike compounds. Another example is that analytes in GC or HPLC methods must fall within the established retention time windows in order to be used to develop acceptance criteria.

8.7.8 Laboratories are advised to consider the effects of the spiking concentration on matrix spike performance criteria, and to avoid censoring of data. As noted in Sec. 8.5.4, the acceptance criteria for matrix spike recovery and precision are often a function of the spike concentration used. Therefore, use caution when pooling matrix spike/matrix spike duplicate data for use in establishing acceptance criteria. Not only should the results all be from the same (or very similar) matrix, but the spiking levels should also be approximately the same (within a factor of 2).

Similarly, the matrix spike and surrogate results should all be generated using the same set of extraction, cleanup, and analysis techniques. For example, do not mix results from solid samples extracted by ultrasonic extraction with those extracted by Soxhlet.

8.7.9 Another common error in developing acceptance criteria is to discard data that do not meet a preconceived notion of acceptable performance. This results in a censored data set, which, when used to develop acceptance criteria, will lead to unrealistically narrow criteria. Remember that for a 95% confidence interval, 1 out of every 20 observations likely will still fall outside the limits.

While professional judgement is important in evaluating data to be used to develop acceptance criteria, do not discard specific results simply because they do not meet one's expectations. Rather, employ a statistical test for outlier values, or at least calculate the acceptance limits both with and without the results that are considered suspect and observe the effect of deleting suspect data.

8.7.10 In-house QC limits must be examined for reasonableness. It is not EPA's intent to legitimize poor recoveries that are due to the incorrect choice of methods or spiking levels. In-house limits also should be compared with the objectives of specific analyses. For example, recovery limits (for surrogates, MS, MSD, LCS, etc.) that include allowance for a relatively high positive bias (e.g., 70 - 170%) may be appropriate for determining that an analyte is <u>not</u> present in a sample. However, they would be less appropriate for the analysis of samples near but below a regulatory limit, because of the potential high bias.

It may be useful to compare QC limits generated in the laboratory to the performance data that may be listed in specific determinative methods. However, the analyst must be aware that performance data generated from multiple-laboratory data tend to be significantly wider than those generated from single-laboratory data. In addition, comparisons between in-house limits and those from other sources should generally focus more on the accuracy (recovery) limits of single analyses rather than the precision limits. For example, a mean recovery closer to 100% is generally preferred, even if the ±3 standard deviation range is slightly wider, because those limits indicate that the result is likely closer to the "true value." In contrast, the precision range provides an indication of the results that might be expected from repeated analyses of the same sample.

8.8 It is recommended that the laboratory adopt additional quality assurance practices for use with these methods. The specific practices that are most productive depend upon the needs of the laboratory, the nature of the samples, and project-specific requirements. Field duplicates may be analyzed to assess the precision of the environmental measurements. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as gas chromatography with a dissimilar column, specific element detector, or mass spectrometer (selected ion monitoring or full scan) must be used. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

9.0 METHOD PERFORMANCE

9.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero. The MDL concentrations listed in the SW-846 analytical methods generally were obtained using organic-free reagent water. Similar results were achieved using representative wastewaters. The MDL actually achieved in a given analysis will vary depending on instrument sensitivity and matrix effects. See Chapter One for more guidance on determination of laboratory-specific MDLs.

9.2 Refer to the determinative methods for method performance information.

10.0 REFERENCES

For further information regarding these methods, review Methods 3500, 3600, 5000, and Chapter One.

METHOD 8330

NITROAROMATICS AND NITRAMINES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

1.0 SCOPE AND APPLICATION

1.1 Method 8330 is intended for the trace analysis of explosives residues by high performance liquid chromatography using a UV detector. This method is used to determine the concentration of the following compounds in a water, soil, or sediment matrix:

Compound	Abbreviation	CAS No[a]
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine	HMX	2691-41-0
Hexahydro-1,3,5-trinitro-1,3,5-triazine	RDX	121-82-4
1,3,5-Trinitrobenzene	1,3,5-TNB	99-35-4
1,3-Dinitrobenzene	1,3-DNB	99-65-0
Methyl-2,4,6-trinitrophenylnitramine	Tetryl	479-45-8
Nitrobenzene	NB	98-95-3
2,4,6-Trinitrotoluene	2,4,6-TNT	118-96-7
4-Amino-2,6-dinitrotoluene	4-Am-DNT	1946-51-0
2-Amino-4, 6-dinitrotoluene	2-Am-DNT	355-72-78-2
2,4-Dinitrotoluene	2,4-DNT	121-14-2
2,6-Dinitrotoluene	2,6-DNT	606-20-2
2-Nitrotoluene	2-NT	88-72-2
3-Nitrotoluene	3-NT	99-08-1
4-Nitrotoluene	4-NT	99-99-0

a Chemical Abstracts Service Registry number

1.2 Method 8330 provides a salting-out extraction procedure for low concentration (parts per trillion, or nanograms per liter) of explosives residues in surface or ground water. Direct injection of diluted and filtered water samples can be used for water samples of higher concentration (See Table 1).

1.3 All of these compounds are either used in the manufacture of explosives or are the degradation products of compounds used for that purpose. When making stock solutions for calibration, treat each explosive compound with caution. See NOTE in Sec. 5.3.1 and Sec. 11 on Safety.

1.4 The estimated quantitation limits (EQLs) of target analytes determined by Method 8330 in water and soil are presented in Table 1.

1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of HPLC, skilled in the interpretation of

chromatograms, and experienced in handling explosive materials. (See Sec. 11.0 on SAFETY.) Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 Method 8330 provides high performance liquid chromatographic (HPLC) conditions for the detection of ppb levels of certain explosives residues in water, soil and sediment matrix. Prior to use of this method, appropriate sample preparation techniques must be used.

2.2 Low-Level Salting-out Method With No Evaporation: Aqueous samples of low concentration are extracted by a salting-out extraction procedure with acetonitrile and sodium chloride. The small volume of acetonitrile that remains undissolved above the salt water is drawn off and transferred to a smaller volumetric flask. It is back-extracted by vigorous stirring with a specific volume of salt water. After equilibration, the phases are allowed to separate and the small volume of acetonitrile residing in the narrow neck of the volumetric flask is removed using a Pasteur pipet. The concentrated extract is diluted 1:1 with reagent grade water. An aliquot is separated on a C-18 reverse phase column, determined at 254 nm, and confirmed on a CN reverse phase column.

2.3 High-level Direct Injection Method: Aqueous samples of higher concentration can be diluted 1/1 (v/v) with methanol or acetonitrile, filtered, separated on a C-18 reverse phase column, determine at 254 nm, and confirmed on a CN reverse phase column. If HMX is an important target analyte, methanol is preferred.

2.4 Soil and sediment samples are extracted using acetonitrile in an ultrasonic bath, filtered and chromatographed as in Sec. 2.3.

3.0 INTERFERENCES

3.1 Solvents, reagents, glassware and other sample processing hardware may yield discrete artifacts and/or elevated baselines, causing misinterpretation of the chromatograms. All of these materials must be demonstrated to be free from interferences.

3.2 2,4-DNT and 2,6-DNT elute at similar retention times (retention time difference of 0.2 minutes). A large concentration of one isomer may mask the response of the other isomer. If it is not apparent that both isomers are present (or are not detected), an isomeric mixture should be reported.

3.3 Tetryl decomposes rapidly in methanol/water solutions, as well as with heat. All aqueous samples expected to contain tetryl should be diluted with acetonitrile prior to filtration and acidified to pH <3. All samples expected to contain tetryl should not be exposed to temperatures above room temperature.

3.4 Degradation products of tetryl appear as a shoulder on the 2,4,6-TNT peak. Peak heights rather than peak areas should be used when tetryl is present in concentrations that are significant relative to the concentration of 2,4,6-TNT.

4.0 APPARATUS AND MATERIALS

4.1 HPLC system

4.1.1 HPLC - equipped with a pump capable of achieving 4000 psi, a 100 µl loop injector and a 254 nm UV detector (Perkin Elmer Series 3, or equivalent). For the low concentration option, the detector must be capable of a stable baseline at 0.001 absorbance units full scale.

4.1.2 Recommended Columns:

4.1.2.1 Primary column: C-18 Reverse phase HPLC column, 25 cm x 4.6 mm (5 µm), (Supelco LC-18, or equivalent).

4.1.2.2 Secondary column: CN Reverse phase HPLC column, 25 cm x 4.6 mm (5 µm), (Supelco LC-CN, or equivalent).

4.1.3 Strip chart recorder.

4.1.4 Digital integrator (optional).

4.1.5 Autosampler (optional).

4.2 Other Equipment

4.2.1 Temperature controlled ultrasonic bath.

4.2.2 Vortex mixer.

4.2.3 Balance, ± 0.0001 g.

4.2.4 Magnetic stirrer with stirring pellets.

4.2.5 Water bath - Heated, with concentric ring cover, capable of temperature control (± 5°C). The bath should be used in a hood.

4.2.6 Oven - Forced air, without heating.

4.3 Materials

4.3.1 High pressure injection syringe - 500 µL, (Hamilton liquid syringe or equivalent).

4.3.2 Disposable cartridge filters - 0.45 µm Teflon filter.

4.3.3 Pipets - Class A, glass, Appropriate sizes.

4.3.4 Pasteur pipets.

4.3.5 Scintillation Vials - 20 mL, glass.

4.3.6 Vials - 15 mL, glass, Teflon-lined cap.

4.3.7 Vials- 40 mL, glass, Teflon-lined cap.

4.3.8 Disposable syringes - Plastipak, 3 mL and 10 mL or equivalent.

4.3.9 Volumetric flasks - Appropriate sizes with ground glass stoppers, Class A.

NOTE: The 100 mL and 1 L volumetric flasks used for magnetic stirrer extraction must be round.

4.3.10 Vacuum desiccator - Glass.

4.3.11 Mortar and pestle - Steel.

4.3.12 Sieve - 30 mesh.

4.3.13 Graduated cylinders - Appropriate sizes.

4.4 Preparation of Materials

4.4.1 Prepare all materials to be used as described in Chapter 4 for semivolatile organics.

5.0 REAGENTS

5.1 Reagent grade inorganic chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lowering the accuracy of the determination.

5.1.1 Acetonitrile, CH_3CN - HPLC grade.

5.1.2 Methanol, CH_3OH - HPLC grade.

5.1.3 Calcium chloride, $CaCl_2$ - Reagent grade. Prepare an aqueous solution of 5 g/L.

5.1.4 Sodium chloride, NaCl, shipped in glass bottles - reagent grade.

5.2 Organic-free reagent water - All references to water in this method refer to organic-free reagent water, as defined in Chapter One.

5.3 Stock Standard Solutions

5.3.1 Dry each solid analyte standard to constant weight in a vacuum desiccator in the dark. Place about 0.100 g (weighed to 0.0001 g) of a single analyte into a 100 mL volumetric flask and dilute to volume with

acetonitrile. Invert flask several times until dissolved. Store in refrigerator at 4°C in the dark. Calculate the concentration of the stock solution from the actual weight used (nominal concentration = 1,000 mg/L). Stock solutions may be used for up to one year.

<u>NOTE</u>: The HMX, RDX, Tetryl, and 2,4,6-TNT are explosives and the neat material should be handled carefully. See SAFETY in Sec. 11 for guidance. HMX, RDX, and Tetryl reference materials are shipped under water. Drying at ambient temperature requires several days. DO NOT DRY AT HEATED TEMPERATURES!

5.4 Intermediate Standards Solutions

5.4.1 If both 2,4-DNT and 2,6-DNT are to be determined, prepare two separate intermediate stock solutions containing (1) HMX, RDX, 1,3,5-TNB, 1,3-DNB, NB, 2,4,6-TNT, and 2,4-DNT and (2) Tetryl, 2,6-DNT, 2-NT, 3-NT, and 4-NT. Intermediate stock standard solutions should be prepared at 1,000 µg/L, in acetonitrile when analyzing soil samples, and in methanol when analyzing aqueous samples.

5.4.2 Dilute the two concentrated intermediate stock solutions, with the appropriate solvent, to prepare intermediate standard solutions that cover the range of 2.5 - 1,000 µg/L. These solutions should be refrigerated on preparation, and may be used for 30 days.

5.4.3 For the low-level method, the analyst must conduct a detection limit study and devise dilution series appropriate to the desired range. Standards for the low level method must be prepared immediately prior to use.

5.5 Working standards

5.5.1 Calibration standards at a minimum of five concentration levels should be prepared through dilution of the intermediate standards solutions by 50% (v/v) with 5 g/L calcium chloride solution (Sec. 5.1.3). These solutions must be refrigerated and stored in the dark, and prepared fresh on the day of calibration.

5.6 Surrogate Spiking Solution

5.6.1 The analyst should monitor the performance of the extraction and analytical system as well as the effectiveness of the method in dealing with each sample matrix by spiking each sample, standard and reagent water blank with one or two surrogates (e.g., analytes not expected to be present in the sample).

5.7 Matrix Spiking Solutions

5.7.1 Prepare matrix spiking solutions in methanol such that the concentration in the sample is five times the Estimated Quantitation Limit (Table 1). All target analytes should be included.

5.8 HPLC Mobile Phase

5.8.1 To prepare 1 liter of mobile phase, add 500 mL of methanol to 500 mL of organic-free reagent water.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 Follow conventional sampling and sample handling procedures as specified for semivolatile organics in Chapter Four.

6.2 Samples and sample extracts must be stored in the dark at 4 C. Holding times are the same as for semivolatile organics.

7.0 PROCEDURE

7.1 Sample Preparation

7.1.1 Aqueous Samples: It is highly recommended that process waste samples be screened with the high-level method to determine if the low level method (1-50 µg/L) is required. Most groundwater samples will fall into the low level method.

7.1.1.1 Low-Level Method (salting-out extraction)

7.1.1.1.1 Add 251.3 g of sodium chloride to a 1 L volumetric flask (round). Measure out 770 mL of a water sample (using a 1 L graduated cylinder) and transfer it to the volumetric flask containing the salt. Add a stir bar and mix the contents at maximum speed on a magnetic stirrer until the salt is completely dissolved.

7.1.1.1.2 Add 164 mL of acetonitrile (measured with a 250 mL graduated cylinder) while the solution is being stirred and stir for an additional 15 minutes. Turn off the stirrer and allow the phases to separate for 10 minutes.

7.1.1.1.3 Remove the acetonitrile (upper) layer (about 8 mL) with a Pasteur pipet and transfer it to a 100 mL volumetric flask (round). Add 10 mL of fresh acetonitrile to the water sample in the 1 L flask. Again stir the contents of the flask for 15 minutes followed by 10 minutes for phase separation. Combine the second acetonitrile portion with the initial extract. The inclusion of a few drops of salt water at this point is unimportant.

7.1.1.1.4 Add 84 mL of salt water (325 g NaCl per 1000 mL of reagent water) to the acetonitrile extract in the 100 mL volumetric flask. Add a stir bar and stir the contents on a magnetic stirrer for 15 minutes, followed by 10 minutes for phase separation. Carefully transfer the acetonitrile phase

to a 10 mL graduated cylinder using a Pasteur pipet. At this stage, the amount of water transferred with the acetonitrile must be minimized. The water contains a high concentration of NaCl that produces a large peak at the beginning of the chromatogram, where it could interfere with the HMX determination.

7.1.1.1.5 Add an additional 1.0 mL of acetonitrile to the 100 mL volumetric flask. Again stir the contents of the flask for 15 minutes, followed by 10 minutes for phase separation. Combine the second acetonitrile portion with the initial extract in the 10 mL graduated cylinder (transfer to a 25 mL graduated cylinder if the volume exceeds 5 mL). Record the total volume of acetonitrile extract to the nearest 0.1 mL. (Use this as the volume of total extract [V_t] in the calculation of concentration after converting to µL). The resulting extract, about 5 - 6 mL, is then diluted 1:1 with organic-free reagent water (with pH <3 if tetryl is a suspected analyte) prior to analysis.

7.1.1.1.6 If the diluted extract is turbid, filter it through a 0.45 - µm Teflon filter using a disposable syringe. Discard the first 0.5 mL of filtrate, and retain the remainder in a Teflon-capped vial for RP-HPLC analysis as in Sec. 7.4.

7.1.1.2 High-Level Method

7.1.1.2.1 Sample filtration: Place a 5 mL aliquot of each water sample in a scintillation vial, add 5 mL of acetonitrile, shake thoroughly, and filter through a 0.45-µm Teflon filter using a disposable syringe. Discard the first 3 mL of filtrate, and retain the remainder in a Teflon-capped vial for RP-HPLC analysis as in Sec. 7.4. HMX quantitation can be improved with the use of methanol rather than acetonitrile for dilution before filtration.

7.1.2 Soil and Sediment Samples

7.1.2.1 Sample homogenization: Dry soil samples in air at room temperature or colder to a constant weight, being careful not to expose the samples to direct sunlight. Grind and homogenize the dried sample thoroughly in an acetonitrile-rinsed mortar to pass a 30 mesh sieve.

NOTE: Soil samples should be screened by Method 8515 prior to grinding in a mortar and pestle (See Safety Sec. 11.2).

7.1.2.2 Sample extraction

7.1.2.2.1 Place a 2.0 g subsample of each soil sample in a 15 mL glass vial. Add 10.0 mL of acetonitrile, cap with

Teflon-lined cap, vortex swirl for one minute, and place in a cooled ultrasonic bath for 18 hours.

7.1.2.2.2 After sonication, allow sample to settle for 30 minutes. Remove 5.0 mL of supernatant, and combine with 5.0 mL of calcium chloride solution (Sec. 5.1.3) in a 20 mL vial. Shake, and let stand for 15 minutes.

7.1.2.2.3 Place supernatant in a disposable syringe and filter through a 0.45-µm Teflon filter. Discard first 3 mL and retain remainder in a Teflon-capped vial for RP-HPLC analysis as in Sec. 7.4.

7.2 Chromatographic Conditions (Recommended)

Primary Column:	C-18 reverse phase HPLC column, 25-cm x 4.6-mm, 5 µm, (Supelco LC-18 or equivalent).
Secondary Column:	CN reverse phase HPLC column, 25-cm x 4.6-mm, 5 µm, (Supelco LC-CN or equivalent).
Mobile Phase:	50/50 (v/v) methanol/organic-free reagent water.
Flow Rate:	1.5 mL/min
Injection volume:	100-µL
UV Detector:	254 nm

7.3 Calibration of HPLC

7.3.1 All electronic equipment is allowed to warm up for 30 minutes. During this period, at least 15 void volumes of mobile phase are passed through the column (approximately 20 min at 1.5 mL/min) and continued until the baseline is level at the UV detector's greatest sensitivity.

7.3.2 Initial Calibration. Injections of each calibration standard over the concentration range of interest are made sequentially into the HPLC in random order. Peak heights or peak areas are obtained for each analyte. Experience indicates that a linear calibration curve with zero intercept is appropriate for each analyte. Therefore, a response factor for each analyte can be taken as the slope of the best-fit regression line.

7.3.3 Daily Calibration. Analyze midpoint calibration standards, at a minimum, at the beginning of the day, singly at the midpoint of the run, and singly after the last sample of the day (assuming a sample group of 10 samples or less). Obtain the response factor for each analyte from the mean peak heights or peak areas and compare it with the response factor obtained for the initial calibration. The mean response factor for the

daily calibration must agree within ±15% of the response factor of the initial calibration. The same criteria is required for subsequent standard responses compared to the mean response of the triplicate standards beginning the day. If this criterion is not met, a new initial calibration must be obtained.

7.4 HPLC Analysis

7.4.1 Analyze the samples using the chromatographic conditions given in Sec. 7.2. All positive measurements observed on the C-18 column must be confirmed by injection onto the CN column.

7.4.2 Follow Sec. 7.0 in Method 8000 for instructions on the analysis sequence, appropriate dilutions, establishing daily retention time windows, and identification criteria. Include a mid-level standard after each group of 10 samples in the analysis sequence. If column temperature control is not employed, special care must be taken to ensure that temperature shifts do not cause peak misidentification.

7.4.3 Table 2 summarizes the estimated retention times on both C-18 and CN columns for a number of analytes analyzable using this method. An example of the separation achieved by Column 1 is shown in Figure 1.

7.4.4 Record the resulting peak sizes in peak heights or area units. The use of peak heights is recommended to improve reproducibility of low level samples.

7.4.5 Calculation of concentration is covered in Sec. 7.0 of Method 8000.

8.0 QUALITY CONTROL

8.1 Refer to Chapter One for specific quality control procedures. Quality control to validate sample extraction is covered in Method 3500.

8.2 Quality control required to validate the HPLC system operation is found in Method 8000, Sec. 8.0.

8.3 Prior to preparation of stock solutions, acetonitrile, methanol, and water blanks should be run to determine possible interferences with analyte peaks. If the acetonitrile, methanol, or water blanks show contamination, a different batch should be used.

9.0 METHOD PERFORMANCE

9.1 Table 3 presents the single laboratory precision based on data from the analysis of blind duplicates of four spiked soil samples and four field contaminated samples analyzed by seven laboratories.

9.2 Table 4 presents the multilaboratory error based on data from the analysis of blind duplicates of four spiked soil samples and four field contaminated samples analyzed by seven laboratories.

9.3 Table 5 presents the multilaboratory variance of the high concentration method for water based on data from nine laboratories.

9.4 Table 6 presents multilaboratory recovery data from the analysis of spiked soil samples by seven laboratories.

9.5 Table 7 presents a comparison of method accuracy for soil and aqueous samples (high concentration method).

9.6 Table 8 contains precision and accuracy data for the salting-out extraction method.

10.0 REFERENCES

1. Bauer, C.F., T.F. Jenkins, S.M. Koza, P.W. Schumacher, P.H. Miyares and M.E. Walsh (1989). Development of an analytical method for the determination of explosive residues in soil. Part 3. Collaborative test results and final performance evaluation. USA Cold Regions Research and Engineering Laboratory, CRREL Report 89-9.

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7. Jenkins, T.F., P.W. Schumacher, M.E. Walsh and C.F. Bauer (1988b) Development of an analytical method for the determination of explosive

residues in soil. Part II: Further development and ruggedness testing. USA Cold Regions Research and Engineering Laboratory, CRREL Report 88-8.

8. Leggett, D.C., T.F. Jenkins and P.H. Miyares (1990) Salting-out solvent extraction for preconcentration of neutral polar organic solutes from water. Analytical Chemistry, 62: 1355-1356.

9. Miyares, P.H. and T.F. Jenkins (1990) Salting-out solvent extraction for determining low levels of nitroaromatics and nitramines in water. USA Cold Regions Research and Engineering Laboratory, Special Report 90-30.

11.0 SAFETY

11.1 Standard precautionary measures used for handling other organic compounds should be sufficient for the safe handling of the analytes targeted by Method 8330. The only extra caution that should be taken is when handling the analytical standard neat material for the explosives themselves and in rare cases where soil or waste samples are highly contaminated with the explosives. Follow the note for drying the neat materials at ambient temperatures.

11.2 It is advisable to screen soil or waste samples using Method 8515 to determine whether high concentrations of explosives are present. Soil samples as high as 2% 2,4,6-TNT have been safely ground. Samples containing higher concentrations should not be ground in the mortar and pestle. Method 8515 is for 2,4,6-TNT, however, the other nitroaromatics will also cause a color to be developed and provide a rough estimation of their concentrations. 2,4,6-TNT is the analyte most often detected in high concentrations in soil samples. Visual observation of a soil sample is also important when the sample is taken from a site expected to contain explosives. Lumps of material that have a chemical appearance should be suspect and not ground. Explosives are generally a very finely ground grayish-white material.

TABLE 1
ESTIMATED QUANTITATION LIMITS

Compounds	Water (µg/L) Low-Level	Water (µg/L) High-Level	Soil (mg/kg)
HMX	-	13.0	2.2
RDX	0.84	14.0	1.0
1,3,5-TNB	0.26	7.3	0.25
1,3-DNB	0.11	4.0	0.25
Tetryl	-	4.0	0.65
NB	-	6.4	0.26
2,4,6-TNT	0.11	6.9	0.25
4-Am-DNT	0.060	-	-
2-Am-DNT	0.035	-	-
2,6-DNT	0.31	9.4	0.26
2,4-DNT	0.020	5.7	0.25
2-NT	-	12.0	0.25
4-NT	-	8.5	0.25
3-NT	-	7.9	0.25

TABLE 2
RETENTION TIMES AND CAPACITY FACTORS ON LC-18 AND LC-CN COLUMNS

	Retention time (min)		Capacity factor (k)*	
Compound	LC-18	LC-CN	LC-18	LC-CN
HMX	2.44	8.35	0.49	2.52
RDX	3.73	6.15	1.27	1.59
1,3,5-TNB	5.11	4.05	2.12	0.71
1,3-DNB	6.16	4.18	2.76	0.76
Tetryl	6.93	7.36	3.23	2.11
NB	7.23	3.81	3.41	0.61
2,4,6-TNT	8.42	5.00	4.13	1.11
4-Am-DNT	8.88	5.10	4.41	1.15
2-Am-DNT	9.12	5.65	4.56	1.38
2,6-DNT	9.82	4.61	4.99	0.95
2,4-DNT	10.05	4.87	5.13	1.05
2-NT	12.26	4.37	6.48	0.84
4-NT	13.26	4.41	7.09	0.86
3-NT	14.23	4.45	7.68	0.88

* Capacity factors are based on an unretained peak for nitrate at 1.71 min on LC-18 and at 2.00 min on LC-CN.

TABLE 3
SINGLE LABORATORY PRECISION OF METHOD FOR SOIL SAMPLES

	Spiked Soils			Field-Contaminated Soils		
	Mean Conc. (mg/kg)	SD	%RSD	Mean Conc. (mg/kg)	SD	%RSD
HMX	46	1.7	3.7	14	1.8	12.8
				153	21.6	14.1
RDX	60	1.4	2.3	104	12	11.5
				877	29.6	3.4
1,3,5-TNB	8.6	0.4	4.6	2.8	0.2	7.1
	46	1.9	4.1	72	6.0	8.3
1,3-DNB	3.5	0.14	4.0	1.1	0.11	9.8
Tetryl	17	3.1	17.9	2.3	0.41	18.0
2,4,6-TNT	40	1.4	3.5	7.0	0.61	9.0
				669	55	8.2
2,4-DNT	5.0	0.17	3.4	1.0	0.44	42.3

TABLE 4
MULTILABORATORY ERROR OF METHOD FOR SOIL SAMPLES

	Spiked Soils			Field-Contaminated Soils		
	Mean Conc. (mg/kg)	SD	%RSD	Mean Conc. (mg/kg)	SD	%RSD
HMX	46	2.6	5.7	14 153	3.7 37.3	26.0 24.0
RDX	60	2.6	4.4	104 877	17.4 67.3	17.0 7.7
1,3,5-TNB	8.6 46	0.61 2.97	7.1 6.5	2.8 72	0.23 8.8	8.2 12.2
1,3-DNB	3.5	0.24	6.9	1.1	0.16	14.5
Tetryl	17	5.22	30.7	2.3	0.49	21.3
2,4,6-TNT	40	1.88	4.7	7.0 669	1.27 63.4	18.0 9.5
2,4-DNT	5.0	0.22	4.4	1.0	0.74	74.0

TABLE 5
MULTILABORATORY VARIANCE OF METHOD FOR WATER SAMPLES[a]

Compounds	Mean Conc. (μg/L)	SD	%RSD
HMX	203	14.8	7.3
RDX	274	20.8	7.6
2,4-DNT	107	7.7	7.2
2,4,6-TNT	107	11.1	10.4

[a] Nine Laboratories

TABLE 6
MULTILABORATORY RECOVERY DATA FOR SPIKED SOIL SAMPLES

	Concentration (μg/g)						
Laboratory	HMX	RDX	1,3,5-TNB	1,3-DNB	Tetryl	2,4,6-TNT	2,4-DNT
1	44.97	48.78	48.99	49.94	32.48	49.73	51.05
3	50.25	48.50	45.85	45.96	47.91	46.25	48.37
4	42.40	44.00	43.40	49.50	31.60	53.50	50.90
5	46.50	48.40	46.90	48.80	32.10	55.80	49.60
6	56.20	55.00	41.60	46.30	13.20	56.80	45.70
7	41.50	41.50	38.00	44.50	2.60	36.00	43.50
8	52.70	52.20	48.00	48.30	44.80	51.30	49.10
True Conc	50.35	50.20	50.15	50.05	50.35	50.65	50.05
Mean	47.79	48.34	44.68	47.67	29.24	49.91	48.32
Std Dev	5.46	4.57	3.91	2.09	16.24	7.11	2.78
% RSD	11.42	9.45	8.75	4.39	55.53	14.26	5.76
% Diff*	5.08	3.71	10.91	4.76	41.93	1.46	3.46
Mean % Recovery	95	96	89	95	58	98	96

* Between true value and mean determined value.

TABLE 7
COMPARISON OF METHOD ACCURACY FOR SOIL AND AQUEOUS SAMPLES
(HIGH CONCENTRATION METHOD)

	Recovery (%)	
Analyte	Soil Method*	Aqueous Method**
2,4-DNT	96.0	98.6
2,4,6-TNT	96.8	94.4
RDX	96.8	99.6
HMX	95.4	95.5

* Taken from Bauer et al. (1989), Reference 1.
** Taken from Jenkins et al. (1984), Reference 3.

TABLE 8
PRECISION AND ACCURACY DATA FOR THE SALTING-OUT EXTRACTION METHOD

Analyte	No. of Samples[1]	Precision (% RSD)	Ave. Recovery (%)	Conc. Range (μg/L)
HMX	20	10.5	106	0-1.14
RDX	20	8.7	106	0-1.04
1,3,5-TNB	20	7.6	119	0-0.82
1,3-DNB	20	6.6	102	0-1.04
Tetryl	20	16.4	93	0-0.93
2,4,6-TNT	20	7.6	105	0-0.98
2-Am-DNT	20	9.1	102	0-1.04
2,4-DNT	20	5.8	101	0-1.01
1,2-NT	20	9.1	102	0-1.07
1,4-NT	20	18.1	96	0-1.06
1,3-NT	20	12.4	97	0-1.23

[1]Reagent water

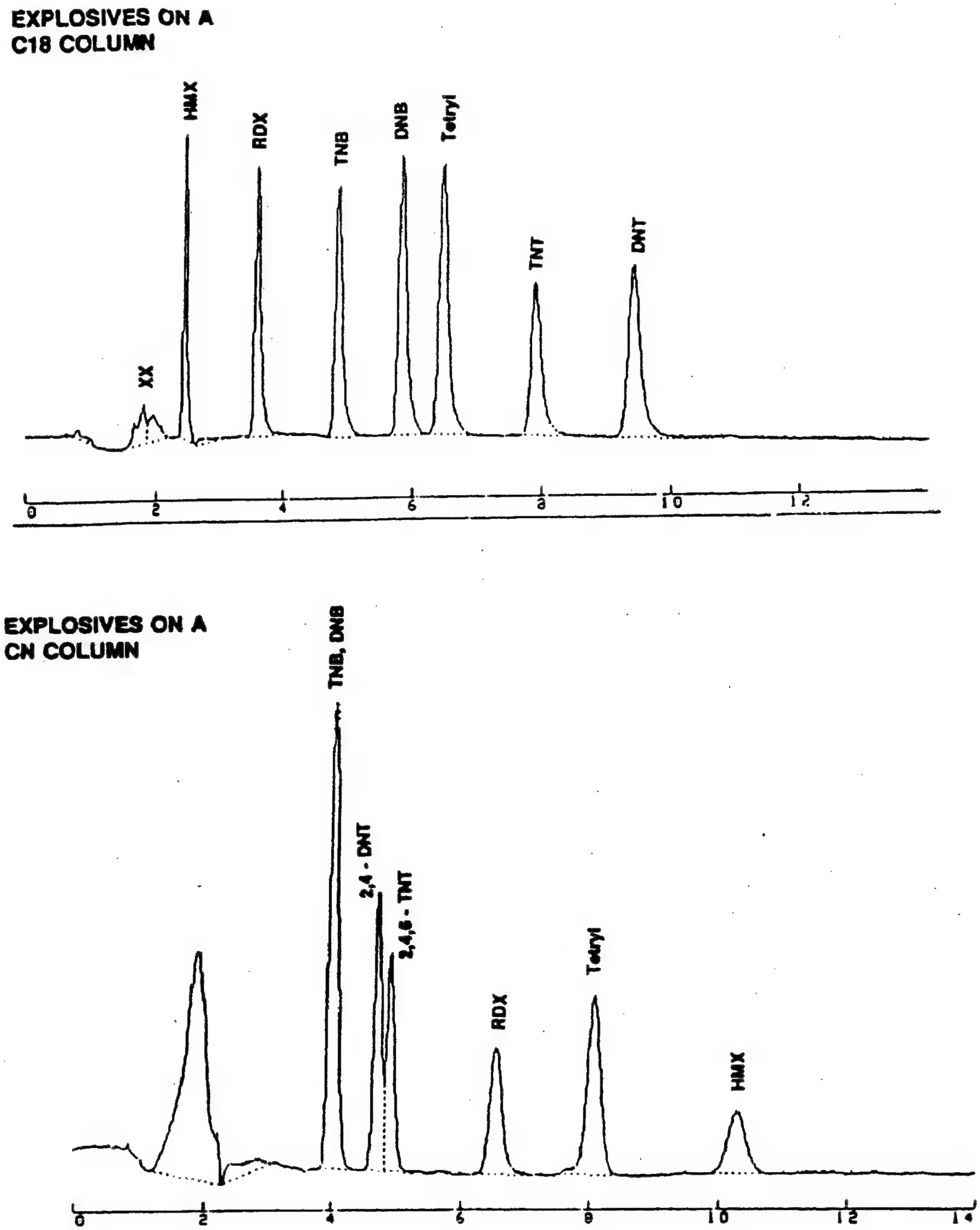
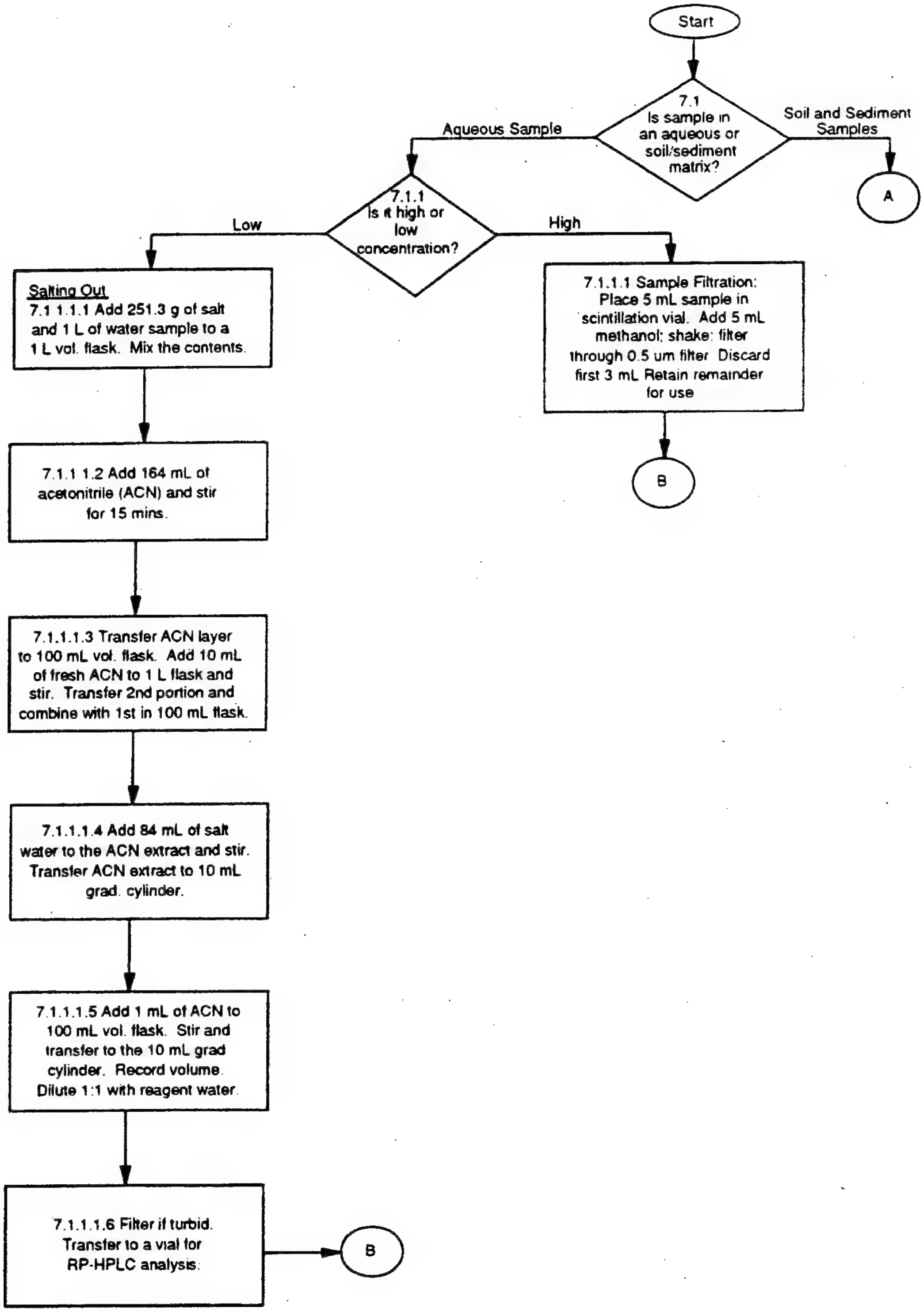
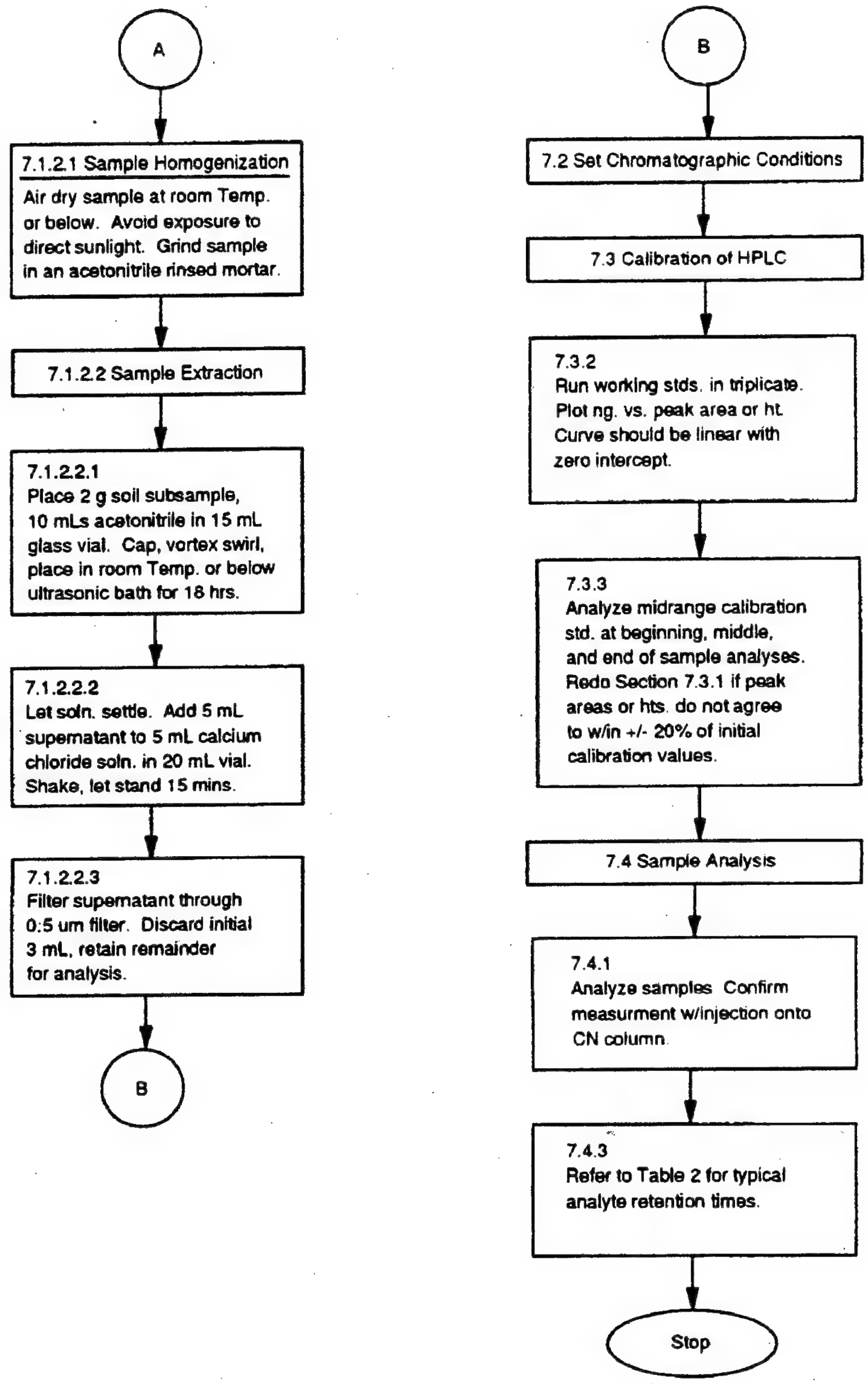


FIGURE 1
CHROMATOGRAMS FOR COLUMNS DESCRIBED IN Sec. 4.1.2.
COURTESY OF U.S. ARMY CORPS OF ENGINEERS, OMAHA, NE.


Start
7.1
Is sample in an aqueous or soil/sediment matrix?
Aqueous Sample
Soil and Sediment Samples
A
7.1.1
Is it high or low concentration?
Low
High
Salting Out
7.1.1.1.1 Add 251.3 g of salt and 1 L of water sample to a 1 L vol. flask. Mix the contents.
7.1.1.1.2 Add 164 mL of acetonitrile (ACN) and stir for 15 mins.
7.1.1.1.3 Transfer ACN layer to 100 mL vol. flask. Add 10 mL of fresh ACN to 1 L flask and stir. Transfer 2nd portion and combine with 1st in 100 mL flask.
7.1.1.1.4 Add 84 mL of salt water to the ACN extract and stir. Transfer ACN extract to 10 mL grad. cylinder.
7.1.1.1.5 Add 1 mL of ACN to 100 mL vol. flask. Stir and transfer to the 10 mL grad cylinder. Record volume. Dilute 1:1 with reagent water.
7.1.1.1.6 Filter if turbid. Transfer to a vial for RP-HPLC analysis.
B
7.1.1.1 Sample Filtration: Place 5 mL sample in scintillation vial. Add 5 mL methanol; shake; filter through 0.5 um filter. Discard first 3 mL. Retain remainder for use
B


A
7.1.2.1 Sample Homogenization
Air dry sample at room Temp. or below. Avoid exposure to direct sunlight. Grind sample in an acetonitrile rinsed mortar.
7.1.2.2 Sample Extraction
7.1.2.2.1
Place 2 g soil subsample, 10 mLs acetonitrile in 15 mL glass vial. Cap, vortex swirl, place in room Temp. or below ultrasonic bath for 18 hrs.
7.1.2.2.2
Let soln. settle. Add 5 mL supernatant to 5 mL calcium chloride soln. in 20 mL vial. Shake, let stand 15 mins.
7.1.2.2.3
Filter supernatant through 0:5 um filter. Discard initial 3 mL, retain remainder for analysis.
B
B
7.2 Set Chromatographic Conditions
7.3 Calibration of HPLC
7.3.2
Run working stds. in triplicate. Plot ng. vs. peak area or ht. Curve should be linear with zero intercept.
7.3.3
Analyze midrange calibration std. at beginning, middle, and end of sample analyses. Redo Section 7.3.1 if peak areas or hts. do not agree to w/in +/- 20% of initial calibration values.
7.4 Sample Analysis
7.4.1
Analyze samples Confirm measurment w/injection onto CN column.
7.4.3
Refer to Table 2 for typical analyte retention times.
Stop

Appendix E

Katalyst Method 8330 Standard Operating Procedures and Reporting Limits

Table C-1
Summary of Laboratory Accuracy and Precision
(Interim Remedial Action at Landfills 6 and 7)

Analysis	Parameter	QC Type	Water Accuracy	Water Precision	Soil Accuracy	Soil Precision
	2-Fluorobiphenyl	SUR	43 - 116	n/a	30 - 115	n/a
	2-Fluorophenol	SUR	21 - 100	n/a	25 - 151	n/a
Pesticides/ PCB	Aldrin	TRG	45 - 133	30	39 - 151	30
	4,4'-DDT	TRG	61 - 130	30	38 - 159	30
	Dieldrin	TRG	74 - 136	30	42 - 153	30
	Endrin	TRG	74 - 141	30	37 - 173	30
	Gamma-BHC (Lindane)	TRG	72 - 130	30	36 - 156	30
	Heptachlor	TRG	55 - 128	30	27 - 166	30
	PCB 1016	TRG	56 - 119	30	35 - 155	30
	PCB 1260	TRG	34 - 119	30	46 - 134	30
	Decachlorobiphenyl (DCB)	SUR	47 - 148	n/a	45 - 127	n/a
	Tetrachloro-m-xylene (TCMX)	SUR	52 - 127	n/a	39 - 119	n/a
Herbicides	2,4,5-TP (silvex)	TRG	45 - 136	30	16 - 132	30
	2,4-D	TRG	13 - 135	30	9 - 117	30
	Dicamba	TRG	26 - 145	30	45 - 125	30
	2,4-Dichlorophenylacetic acid	SUR	30 - 130	n/a	30 - 130	n/a
Explosives	1,3-DNB	TRG	17 - 97	30	33 - 136	30
	4-Am-DNT	TRG	19 - 119	30	84 - 156	30
	2-Am-DNT	TRG	23 - 98	30	50 - 93	30
	2,4-DNT	TRG	15 - 98	30	32 - 134	30
	2,6-DNT	TRG	17 - 95	30	55 - 89	30

TITLE: DETERMINATION OF EXPLOSIVES IN WATER, WASTE AND SOIL BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY ACCORDING TO SW846 METHOD 8330

Reviewed by: (QA/QC Officer) Michael Travis (KDS)

Reviewed by: (Operations Manager) Barbara Beark

1. PURPOSE

The purpose of this Standard Operating Procedure (SOP) is to describe the method used to determine certain nitro-aromatic explosives and explosive precursors in water and soil samples by high pressure liquid chromatography employing a UV detector.

2. SCOPE AND APPLICATION

Method 8330 employs reverse-phase high performance liquid chromatography (HPLC) for the ppb detection of various nitro-aromatic and nitramine explosive residues in water, soil, and sediment matrices. All compound identifications are supported by confirmation on a reverse-phase cyano column. The target analytes appropriate to Method 8330 are listed below:

Method 8330 Target Analytes

1,3-Dinitrobenzene
2,4-Dinitrotoluene
2,6-Dinitrotoluene
HMX (octahydro-1,3,5,7-tetranitro-s-tetrazocine)
Nitrobenzene
RDX (hexahydro-1,3,5-trinitro-s-triazine)
Tetryl (N-methyl-N,2,4,6-tetranitrobenzenamine)
1,3,5-Trinitrobenzene
2,4,6-Trinitrotoluene
4-Amino-2,6-Dinitrotoluene
2-Amino-4,6-Dinitrotoluene
2-Nitrotoluene
3-Nitrotoluene
4-Nitrotoluene

4.2 INSTRUMENTATION AND INSTRUMENTAL CONDITIONS

PRIMARY ANALYSIS

4.2.1 HPLC - Waters 600E HPLC with Model 712 autosampler.

4.2.2 Detector: Waters 484 variable wavelength UV detector set at 230-250 nm.

4.2.3 Column: Phenomenex ODS (octadecylsilane), reverse-phase column, 25 cm length x 4.6 mm I.D., 5 micrometers (μm) particle size , Phenomenex Inc. Torrance, Ca.).

4.2.4 Mobile phase: Isocratic, 45% methanol/ 55% water (v/v).

4.2.5 Flow rate: 0.8 mL/min.

4.2.6 Injection volume: 400 μL.

4.2.7 Temperature: 26E

CONFIRMATION ANALYSIS

4.2.8 Waters Model 500E HPLC with Waters 712 autosampler or equivalent.

4.2.9 Detector: Waters 484 at 250 nm.

4.2.10 Column: Phenomenex Cyano

4.2.11 Mobile phase: Isocratic, 50/50 (v/v) methanol/water.

4.2.12 Flow rate: 1 mL/min..

4.2.13 Injection volume: 250 μL.

4.2.14 Temperature: 26EC

PRIMARY ANALYSIS

4.3 REAGENTS

4.3.1 Methanol (HPLC grade - American Burdick & Jackson, McGaw Park, IL).

4.3.2 Water (ASTM Type II/HPLC grade - American Burdick & Jackson, McGaw Park, IL).

4.3.3 Acetonitrile (HPLC grade - American Burdick & Jackson, McGaw Park, IL).

4.3.4 Acetone (HPLC grade - American Burdick & Jackson, McGaw Park, IL).

4.4 STANDARDS

The stock standard used for target compound identification and calibration is supplied by Accustandard (Cat.# M-8330R) containing all analytes at a concentration of 1 mg/mL. The surrogate, 3,4-DNT, is also supplied by Accustandard (Cat.# M-8330-IS).

5.0 METHOD INTERFERENCES

Any materials which co-extracted from waters, soils, and sediments, coelute with the explosives through the HPLC column, and which absorb ultraviolet radiation at 250 nm may cause interferences. Carryover from analysis of a highly contaminated sample can result in apparent contamination of the succeeding samples analyzed. Such contamination is often manifested by the presence of unusually broad chromatographic peaks nested among narrower peaks. This interference is minimized by analyzing apparently heavily contaminated samples at the end of a run, or running blanks after heavily contaminated samples until carryover is removed, and/or rinsing the system with a mobile phase containing a high proportion of organic modifier until the contamination is removed.

8.0 STANDARD PREPARATION

Table 8-1 summarizes the concentration of stock solutions and preparation of intermediate calibration standards. Table 8-2 summarizes the preparation and concentration of calibration standards used for initial and daily calibration. To summarize the information presented, a stock standard ampule containing all target analytes at a concentration of 1.0 Fg/mL, and a separate stock standard ampule containing the surrogate, 3,4-DNT, at 1.0 Fg/mL are utilized. An intermediate standard is prepared for the target analytes by diluting 1 mL of the ampule to 10 mL of acetonitrile. The 3,4-DNT intermediate is prepared by addition of 1 mL of the ampule to 10 mL of acetonitrile. The calibration standards are prepared by dilution of the intermediate stock to have a 25% acetonitrile concentration to match the acetonitrile concentrations in the final sample extracts.

8.1 Preparation of Standards

Table 8-1 presents the procedure for preparing stock solutions, combined intermediate stock solutions. For initial calibration, Standards Level 1, Level 2, Level 3, Level 4, Level 5, Level 6, Level 7, Level 8 and a blank described in Table 8-2 are prepared. These solutions are prepared fresh as needed but are not stored for longer than one month at 4° " 2°C (10 mL of standard usually lasts 8 runs). The stock ampules are useable for 12 months. The imtermediate stock solutions are prepared fresh every 6 months. HPLC-grade reagent water is used for dilution to final volumes for calibration standards (acetonitrile concentrations may not be greater than 30%). Daily calibration standards used are Levels 5, 6, and 7, and a blank as outlined in Table 8-2.

Table 8-2. Preparation of Initial Calibration Standards

Standard	Dilute from Standard	Volume (mL)	Final Volume (mL)	Analyte Concentration (Fg/mL)
Level 8	Int. Stock	0.20	10	2.0
Level 7	Int. Stock	0.10	10	1.0
Level 6	Level 7	2.5	5	0.5
Level 5	Level 6	2.0	5	0.2
Level 4	Level 5	2.5	5	0.1
Level 3	Level 4	2.5	5	0.05
Level 2	Level 3	0.8	5	0.008
Level 1	Level 2	2.5	5	0.004

8.2 Stock solutions must be replaced after six months or sooner if degradation of the solution is detected. Working solutions must be replaced after two months or sooner if degradation of the solution is detected.

9.0 PROCEDURE

9.1 Initial Calibration

The initial calibration is performed by analyzing a minimum of 5 of the calibration standards presented in Table 8-2. Instrument conditions and the column are described in Sec. 2-2. The analyte areas are tabulated against their concentrations. If the average calibration factors from the initial calibration display a %RSD $\leq$ 20%, the average calibration factor may be used. A calibration curve may be used if the curve correlation coefficient is $\geq$ 0.995.

9.2 Analytical Sequence

The following analysis sequence is used when analyzing samples using an initial calibration. Preparation of the standards is described in Section 8.

9.3.1 Retention Time Windows

Retention time windows are established by analyzing three standards over the course of 72 hours. The standard deviation of each analyte's retention time is determined, with its window being $\pm$ 3 SD. Absolute retention times for all analytes are established by the first continuing calibration standard of the sequence. If retention times of analytes in CCSs later in the sequence fall outside their windows established by the initial CCS, then corrective action must be taken, and samples reanalyzed which may also have experienced a retention time shift.

9.4 CONFIRMATION ANALYSIS

Analytes that are tentatively identified on the primary column and are above the report limit must be confirmed by analysis on a reverse-phase cyano column. In order to confirm an analyte, a response must be present in the retention windows for the analyte on both the primary column and the confirmation column. The retention windows will be calculated the same way for both columns. Decision points to be made for the identification and reporting of a target analyte are:

9.4.1 Is there a response in the retention window of a target analyte on the primary column and the response is above the reporting limit (RL)?

No. No further action is necessary and the analyte is reported as <RL.

Yes. Analyze the sample extract on the confirmation column.

9.4.2 Is there a response on the confirmation column in the retention window of the target analyte and the response is above the criterion of detection?

No. The analyte is not confirmed and the analyte is reported as <RL adjusted for any dilutions required.

Yes. Report analyte at concentration determined by primary analysis.

CONFIRMATION COLUMN

NB	8.27 min.
13DNB	9.35 min.
135TNB	9.82 min.
Nitrotoluenes	10.48 min.
26DNT	11.42 min.
24DNT	12.02 min.
246TNT	13.10 min.
4A26DT	13.55 min.
2A46DT	14.65 min.
34DNT (Surr.)	15.12 min.
RDX	17.17 min.
Tetryl	25.50 min.
HMX	32.38 min.

10.0 DATA COLLECTION AND CALCULATIONS

10.1 Currently, all data is collected utilizing PE-Nelson Turbo Chrom 4 software. The target responses are transferred to the Laboratory Information Management System, (LIMS), along with any relevant sample information. The concentration is calculated using the regression equation calculated by LIMS. Final sample results are corrected for sample volume or sample weight, extract volume, percent moisture for solid samples, dilution factors and applicable conversion factors.

LIMS Soil Calculation

$$FINALCONC = \frac{CURVCONC * EXTVOL * DL}{SAMPVOL * ((100 - \%MOISTURE) / 100}$$

$$CCSPercentDifference = \frac{R_1 - R_2}{R_1} x100$$

Where: R_1 = theoretical concentration or initial calibration average factor
R_2 = CCS calculated concentration or response factor

.0 QUALITY CONTROL

11.1 An extraction method blank (MB) is analyzed for each extraction batch or 20 samples, whichever is more frequent. This MB must be free of target analytes at $\geq$ the PQL.

11.2 A laboratory control spike is extracted and analyzed with every 20 client samples, or with each extraction batch, whichever is more frequent. Recoveries must be within acceptance ranges given in Attachment 3.

11.3 A matrix spike (MS) and matrix spike duplicate (MSD) are prepared once every 20 samples or once per two weeks, whichever is more frequent. Recoveries should be within acceptance ranges listed in Attachment 3 and must be from a mix different than that used for calibration.

11.4 Control Charts have been established for laboratory control samples and when requested, client or project-specific matrix spike/matrix spike duplicate sets. Control charts are used to determine control limits, and monitor trends or out-of-control situations. After a minimum of 20 results have been obtained for each particular analysis and matrix, the mean result and its standard deviation will be tabulated and calculated. Warning limits are set at +/- 2 standard deviations of the mean and the control limits are set at +/- 3 standard deviations of the mean. Means and standard deviations will be calculated at least annually.

For duplicate analyses (matrix spike/matrix spike duplicate), a control chart is generated using at least 20 duplicate results of a single specific matrix. The mean and standard deviation are calculated on the percent relative percent difference (%RPD) instead of the analytical result. When new ranges are generated, the LIMS system is updated.

If at any time during analysis, the process is out-of-control as a result of an LCS falling outside the control limits, corrective action must be taken and documented unless other evaluations of the system determine that the system is still within control.
The Data Review/Data exception Report is used to document all out-of-control situations

ATTACHMENT 1

Common Acronyms and CAS Numbers for Explosives

ANALYTE	ACRONYM	CAS NUMBER
HMX	HMX	2691-41-0
RDX	RDX	121-84-4
1,3,5-Trinitrobenzene	135TNB	25377-32-6
1,3-Dinitrobenzene	13DNB	99-65-01
Tetryl	TETRYL	479-45-8
Nitrobenzene	NB	98-95-3
2,4,6-Trinitrotoluene	246TNT	118-96-7
4-Amino-2,6-Dinitrotoluene	4A26DT	1946-51-0
2-Amino-4,6-Dinitrotoluene	2A46DT	118-96-7
2,6-Dinitrotoluene	26DNT	606-20-2
2,4-Dinitrotoluene	24DNT	121-14-2
2-Nitrotoluene	2NT	88-72-2
4-Nitrotoluene	4NT	99-99-0
3-Nitrotoluene	3NT	99-08-1

ATTACHMENT 3

Analytes, Precision, and Accuracy Data for Nitroaromatics and Nitroamines by High Performance Liquid Chromatography (HPLC), SW 8330

Parameter	Aqueous		Solid	
	Precision (RPD)	Accuracy (% Recovery)	Precision (RPD)	Accuracy (% Recovery)
HMX	13	84-111	18	80-116
RDX	30	51-111	18	71-107
1,3,5-Trinitrobenzene	28	46-102	25	65-115
1,3-Dinitrobenzene	37	58-132	30	70-130
Methyl-2,4,6-Trinitro-phenylnitramine(Tetryl)	21	67-109	46	65-157
Nitrobenzene	32	44-108	24	72-120
2,4,6-Trinitrotoluene	38	48-124	23	72-118
2,4-Dinitrotoluene	21	60-102	19	68-106
2,6-Dinitrotoluene	26	67-119	44	58-146
o-Nitrotoluene	28	53-109	22	70-114
m-Nitrotoluene	48	40-136	48	40-136
p-Nitrotoluene	26	60-112	26	60-112
4-Amino-2,6-Dinitrotoluene	30	70-130	30	70-130
2-Amino-4,6-Dinitrotoluene	30	70-130	30	70-130
3,4-Dinitroluene	N/A	30-150	N/A	30-150

KATALYST Analytical Technologies, Inc. Reporting Limits For Method 8330		
Compound Name	Water RL (ug/L)	Soil RL (ug/kg)
1,3-Dinitrobenzene	0.35	350
2,4-Dinitrotoluene	0.35	350
2,6-Dinitrotoluene	0.35	350
2-amino-4,6-Dinitrotoluene	0.35	350
4-amino-2,6-Dinitrotoluene	0.35	350
HMX	0.50	500
Nitrobenzene	0.50	500
2-Nitrotoluene	0.50	500
3-Nitrotoluene	0.50	500
4-Nitrotoluene	0.50	500
RDX	0.50	500
Tetryl	0.35	350
1,3,5-Trinitrobenzene	0.35	350
2,4,6-Trinitrotoluene	0.35	350

Appendix F

Boat Safety SOP

STANDARD OPERATING PROCEDURE
WORK ON OR NEAR WATER
INVOLVING THE USE OF BOATS

1.0 PURPOSE

The purpose of this standard operating procedure (SOP) is to provide minimum safety requirements during field activities or operations on or near any body of water where the depth of the water is three feet or greater involving the use of boats with or without outboard motors. The requirements in this SOP are in addition to, not in lieu of, tasks specific safety assessments or Health and Safety Plans.

2.0 SCOPE

This SOP shall apply to all QST Environmental employees who work on, or within five feet of water where the depth of the water is three feet or greater and which utilize a boat with or without an outboard motor on navigable waterways. The requirements of this SOP do not necessarily apply to areas where employees are protected by handrail systems, standard rigid barriers, grating, or other protective systems.

3.0 REFERENCES

3.1 REGULATIONS

- 29 CFR 1926.106 - Working Over or Near Water
- 29 CFR 1926.605 - Marine Operations and Equipment as applicable
- 29 CFR 1918 - Safety and Health Regulations for Long Shoring as applicable
- US Coast Guard Safe Boating Practices

3.2 QST ENVIRONMENTAL HEALTH & SAFETY PROGRAMS & SOPS

- Unknown Chemical Exposure Prevention (UCEP) Program
- Hazard Communication Program
- H&S SOP-160 Health and Safety Recordkeeping

4.0 RESPONSIBILITIES

4.1 OFFICE/LAB/DIVISION MANAGER

4.1.1 Office/Lab/Division Managers shall ensure that this SOP is implemented by assigning the various functions described herein to individuals within their office, lab, or division. In addition to referenced OSHA and US Coast Guard requirements, the Manager shall ensure that provisions of any local or state regulations are also implemented.

4.1.2 Office/Lab/Division Managers shall ensure that employees are informed of their responsibilities under this SOP and that proper training is provided to the affected employees.

4.1.2 Office/Lab/Division Managers shall certify in writing that at least one field team member per boat has had adequate training in accordance to 5.1.1.

4.2 LOCAL HEALTH & SAFETY REPRESENTATIVE (LHSR)/CHEMICAL HYGIENE OFFICER (CHO)

4.2.1 The LHSR/CHO shall ensure records of training are kept in accordance with H&S SOP-160 - Health and Safety Recordkeeping.

4.2.2 LHSR/CHO shall ensure that new or transferred employees subject to these procedures shall receive initial training prior to performing any work covered by this SOP.

4.2.3 The LHSR/CHO shall ensure that the procedures outlined in Section 5 of this SOP are followed.

4.2.4 The LHSR/CHO or Office/Lab/Division Manager shall receive upon request consultation from the Health & Safety Coordinator (HSC) and/or Corporate Health & Safety (CHS).

4.3 PROJECT MANAGER (PM)

4.3.1 The PM shall ensure that this SOP is followed on all QST Environmental projects involving work or operations within the scope of this SOP.

4.3.2 The PM shall ensure that each field team member is trained as required by this SOP.

4.3.3 The PM may, if qualified, provide any training required for the field, either as initial employee training or as additional training needed due to a change(s) in conditions or equipment. To be qualified as a boat safety trainer, the PM must attend a Coast Guard approved boat safety course or be certified in writing by the Office/Lab/Division Manager.

4.3.4 The PM shall ensure that personal flotation protection is used in accordance with applicable regulations.

4.4 FIELD TEAM LEADER (FTL)/ SITE HEALTH & SAFETY OFFICER (SHSO)

4.4.1 The FTL/SHSO shall ensure that the safety requirements outlined in Section 5 of this SOP are implemented, are discussed during site safety meetings, and are documented in a Daily Safety Meeting Checklist that will become part of the project file/record.

4.4.2 The FTL/SHSO shall ensure that the safety requirements outlined in Section 5 of this SOP are followed when QST Environmental projects involve work within the scope of this SOP.

4.4.3 The FTL/SHSO shall ensure that at least one crew member of each boat is trained or certified as described in section 5.1.1.

4.5 FIELD TEAM MEMBER (FTM)

4.5.1 The FTMs shall follow the safety requirements outlined in Section 5 of this SOP and by the FTL/SHSO.

4.5.2 FTMs shall comply with the training requirements of this SOP.

4.5.3 FTMs shall notify the PM/FTL of any problems encountered with the boat, outboard motor, or flotation devices.

4.5.4 FTMs shall be responsible for the proper cleaning, maintaining, care, and storage of personal flotation devices issued.

4.6 HEALTH AND SAFETY COORDINATOR (HSC)

4.6.1 The HSC shall provide guidance and consultation when requested by the Office/Lab/Division Manager or the LHSR/CHO.

4.6.2 The HSC may periodically audit the Office/Lab/Division on behalf of Corporate Health & Safety for compliance with this SOP.

4.7 CORPORATE HEALTH & SAFETY (CHS)

4.7.1 CHS shall ensure that the Office/Lab/Division is periodically audited for compliance with this SOP.

4.7.2 CHS shall provide consultation when requested by the Office/Lab/Division Manager or the LHSR/CHO.

5.0 DESCRIPTION

All employees involved in projects which have work or operations on or near water with a depth of three feet or greater shall be familiar with the potential safety hazards of the specific type of boat and outboard motor being used and demonstrate knowledge of the appropriate safety measures needed to ensure a safe working environment. Training and safety hazards related to projects using boats and outboard motors are discussed below.

5.1 TRAINING

5.1.1 Any employees who may be required to operate a boat on behalf of QST Environmental shall be thoroughly trained in the proper operation of the boat and outboard motor. This training may be provided by the employee's Supervisor, FTL, or a Coast Guard approved boating safety course. For the Supervisor or FTL to be considered adequately trained attendance/certification from a Coast Guard approved course is required. Until the Supervisor or FTL completes an approved boating course the PM, affected individuals, and the Office/Lab/Division Manager must certify in writing that they are sufficiently trained in boat safety. At a minimum, this training shall include and ensure employees are knowledgeable on the following topics:

- Boat handling and elementary seamanship:
  Boat terminology, fueling, crossing wakes, docking/undocking, weather and adverse conditions, man overboard recovery, first aid, anchoring.

- Registration, equipment, and regulations:
  Minimum required safety equipment, accident reporting, responsibility of wake, life preservers, capsizing and/or swamping.

- Marlinespike:
  Lines and knots.

- Knowledgeable in the rules of navigation:
  Right of way, overtaking, meeting, maneuvering, warning signals, and restricted visibility, boat running lights.

- Recognize and comply with requirements of regulatory markers and other aids to navigation:
  Charts, buoys, daymarkers, barges and tows, locks and lockage.

- Engine trouble shooting:
  Fuel system, cooling system, and ignition problems, spare parts, and tools.

- Marine radio use:
  Licenses, operation, calling procedures, distress and safety calls and proper channels.

- Boat trailering:
  Legal requirements, hitch type, tiedowns and safety chains, trailer operation, maneuvering, launching, recovery, and general maintenance.

5.1.2 A daily safety meeting shall be conducted and documented as to the safety concerns pertaining to water conditions and that day's use of boat and outboard motors.

5.2 SAFETY PRECAUTIONS

5.2.1. The buddy system shall be used in all cases where a boat is utilized. A written statement describing alternative procedures affording equivalent protection to affected employees shall be prepared and signed by the Project Manager, Supervisor, and Office/Lab/Division Manager, prior to any variation from the standard buddy system (i.e., minimum two persons per boat); affected personnel shall also sign this document demonstrating their acceptance of these conditions.

5.2.2 No smoking or open flames shall be permitted on any boat with flammable solvents and/or gasoline on board.

5.2.3 Each boat shall be equipped with a first aid kit and fire extinguisher.

5.2.4 Portable fuel tanks shall be removed from vessels prior to fueling whenever feasible. If fuel tanks are not removed from the boat before fueling, then the engine and all electrical equipment shall be turned off during fueling. Any spills must be cleaned up immediately.

5.2.5 All batteries shall be kept in an appropriate type battery box.

5.2.6 Employees not directly involved in launching or recovering boats shall be required to stay clear from any vehicle and trailer being loaded and unloaded to avoid being struck.

5.2.7 Boats shall not be loaded beyond the manufacturer's designated maximum capacity.

5.2.8 Boats shall use navigation lights per the requirements of the US Coast Guard and/or local requirements when underway between sunset and sunrise.

5.2.9 Boats shall have sufficient lighting for work that is being performed at night.

5.3 SAFETY EQUIPMENT

5.3.1 Personal flotation devices shall be the appropriate Coast Guard approved device.

5.3.2 Personal flotation devices shall be worn at all times by operators and passengers of boats when working on or near water where the depth is three feet or greater.

5.3.3 A 30-inch life ring with 90 feet of rope shall be on board or at hand when working on or near water that is three feet or greater in depth.

5.3.4 Hearing protection shall be worn when equipment is in operation unless the FTL has measured and determined the noise level to be less than 85 dBa on a time weighted average basis.

5.3.5 Boats, outboards, and trailers shall be appropriately registered.

5.3.6 When trailers are being towed, the trailer lights shall be in working order, and safety chains and guards shall be properly used.

5.3.7 When in operation, each boat shall have a hand or power operated warning device (e.g., air horn) within reach that is audible at least one half mile away.

5.3.8 Each boat will have a set of appropriate oars or paddles.

5.3.9 The outboard motor kill switch shall be worn by the boat driver during travel under power.

5.3.10 Two-way or marine radios shall be used on navigable waters for communication.

5.4 EMERGENCY SITUATIONS

5.4.1 If a person falls into the water, a life ring shall be thrown to the victim. The victim shall then be retrieved to the edge of the water or boat. If the individual is uninjured, the victim shall be assisted from the water.

5.4.2 If a QST Environmental employee is injured, then first aid may be provided by properly trained personnel. Under no circumstances does QST Environmental require employees to provide first aid to injured personnel. Remember to assess the individual for major head, neck, and back injuries when retrieval is into a boat. Seek and obtain appropriate medical assistance whenever necessary.

5.4.3 Employees assisting a victim shall not place themselves in a situation where they could fall into the water or be injured. Employees shall make every attempt to perform non-entry rescue before considering entering the water to retrieve a victim. Under no circumstances does QST Environmental require any employee to enter the water to perform rescue.

5.4.4 All precautions should be taken to ensure that a victim does not fall back into the water.

5.4.5 Use the marine radio to call for emergency assistance from the Coast Guard.

ATTACHMENT A

SAFE BOATING CHECKLIST

SAFE BOATING CHECKLIST

Project Name : ______________________ Project Number : ______________

Project Manager : ______________________

TRAILER	Yes	No	Comments
Winch and Cable (good and working condition)	__	__	______________
Running Boards	__	__	______________
License Plate	__	__	______________
Trailer Lights (working condition)	__	__	______________
Safety Chains	__	__	______________
Tire Pressure	__	__	______________
Spare Tire	__	__	______________
Grease Bearings	__	__	______________
Transom Saver	__	__	______________
Tie Downs	__	__	______________

BOAT & MOTOR	Yes	No	Comments
Battery (charged)	__	__	______________
Battery Cover	__	__	______________
Power Trim	__	__	______________
Motor Functional	__	__	______________
Navigation Lights	__	__	______________
Motor Oil	__	__	______________

EQUIPMENT	Yes	No	Comments
Spare Prop	__	__	______________
Two Oars	__	__	______________
Anchor with Rope	__	__	______________
Gas Tanks	__	__	______________
Fuel Line	__	__	______________
Drain Plug	__	__	______________
Air Horn	__	__	______________
Boat Cushions (one per person)	__	__	______________
Life Jackets (one per person)	__	__	______________
Life Ring with Rope	__	__	______________
First Aid Kit	__	__	______________
Fire Extinguisher	__	__	______________
Marine Radio	__	__	______________

Signature ____________________________ *Date* ______________

Additional comments may be placed on the back of this Checklist.

Appendix G

Petite Ponar/Eckman Dredge Sampling SOP

Appendix G
Petite Ponar/Eckman Dredge Sampling SOP

Lake sediment samples may be collected utilizing a standard clamshell dredge sampler (e.g., petite ponar or Eckman). In general, the dredge jaws are opened above the water's surface and secured by a locking mechanism. The dredge, which is attached by a rope (⅝-inch or larger), is lowered over the side of the anchored watercraft until it reaches the lake bottom. Once on the bottom, the locking mechanism is released and the jaws close on the bottom substrates while scooping substrate and retaining it within the dredge. The dredge is retrieved to the surface and its contents are emptied into an appropriate decontaminated stainless-steel container. The contents are mixed using a decontaminated stainless-steel trowel to facilitate sample homogeneity. Once mixed, an appropriate amount of substrate is transferred to appropriate sample jars for further analysis. After sample collection, all equipment are cleaned following appropriate decontamination procedures.

The petite ponar bottom grab sampler has an opening of 0.152-meters by 0.152-meters and a maximum volume of 2.4 liters. The Eckman bottom grab sample has a similar opening to the petite ponar and a maximum volume of approximately 3.5 liters.